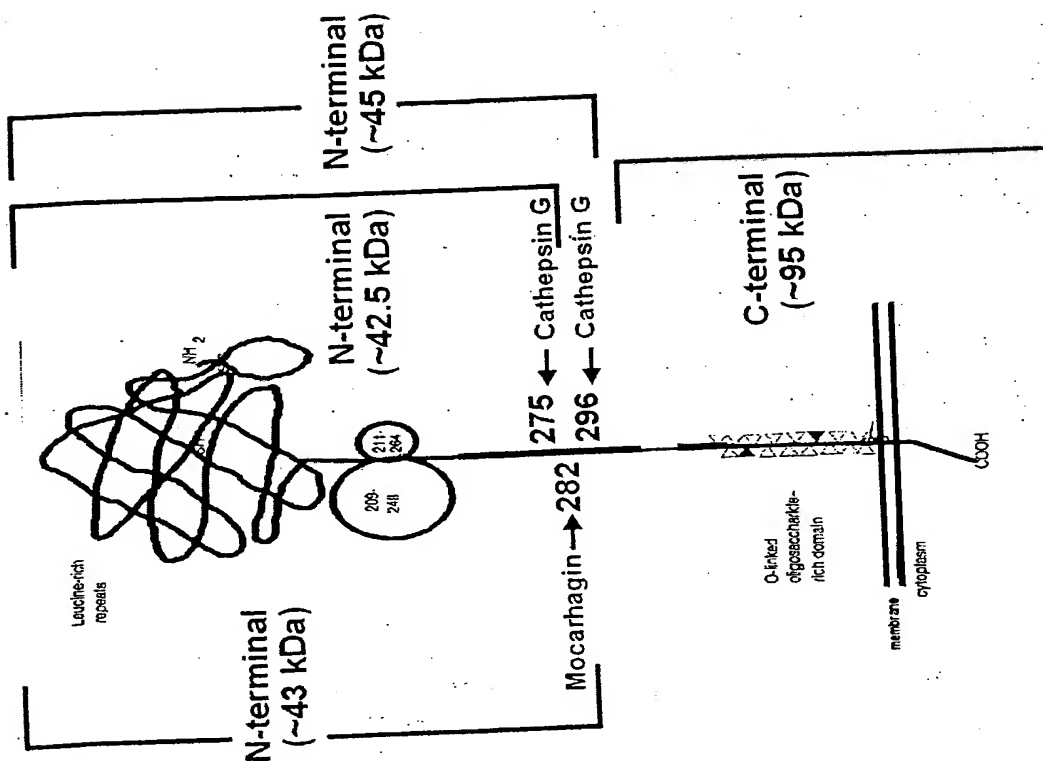




**FIG. 1**

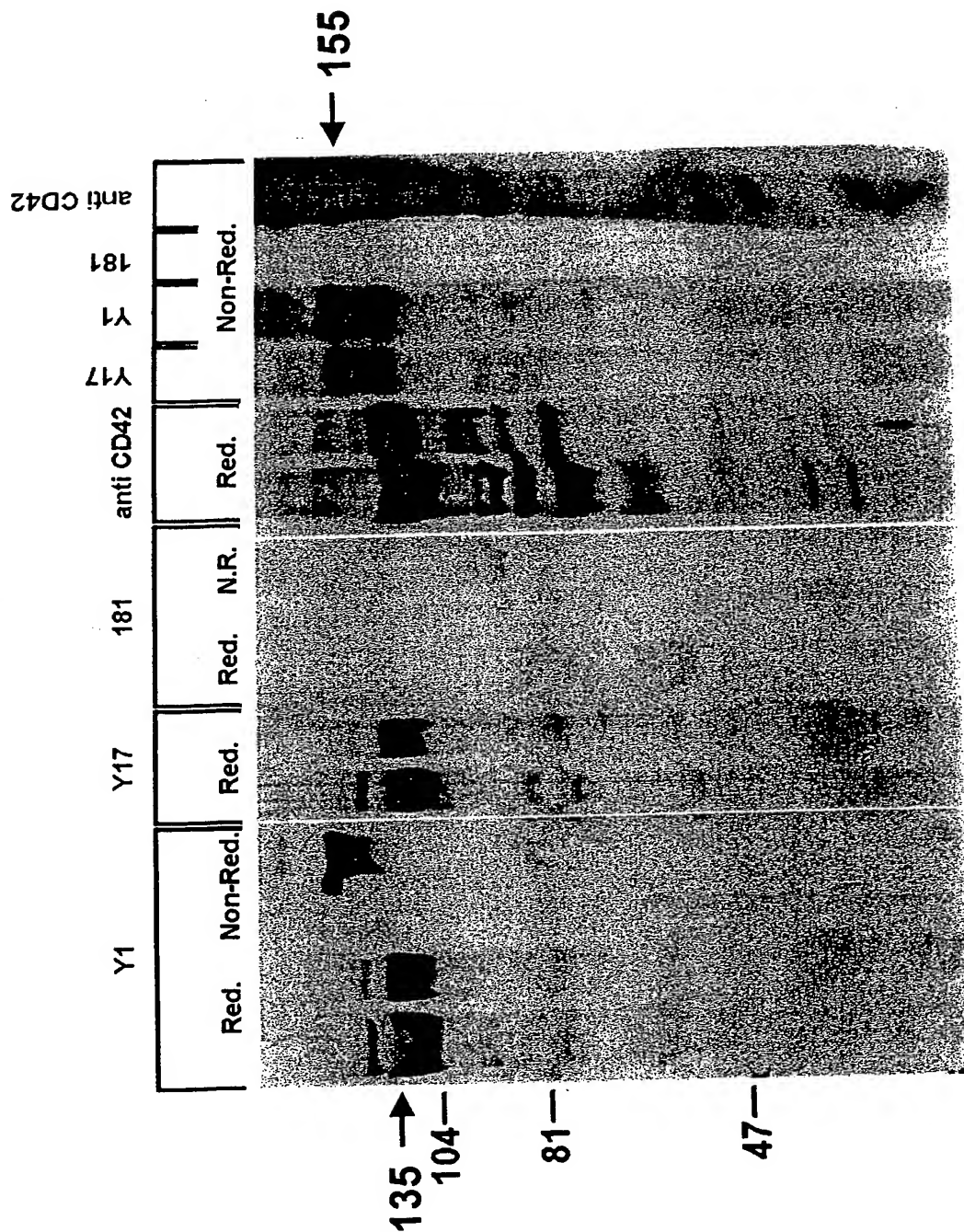
# Cleavage sites of endoproteases on the a-chain of GPIb



#14

FIG. 2

# Binding of Y1 and Y17 to platelets in reduced and non-reduced conditions

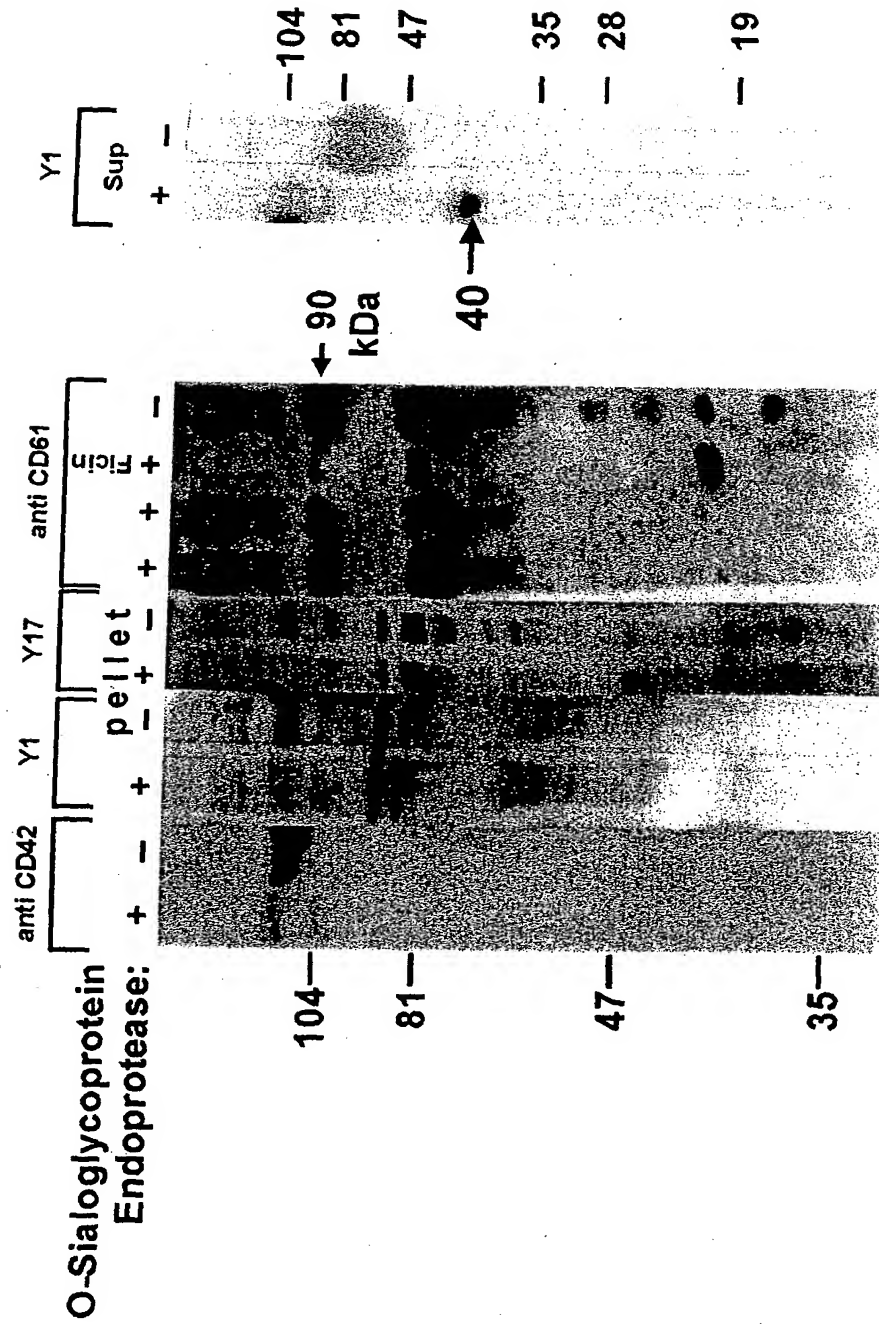
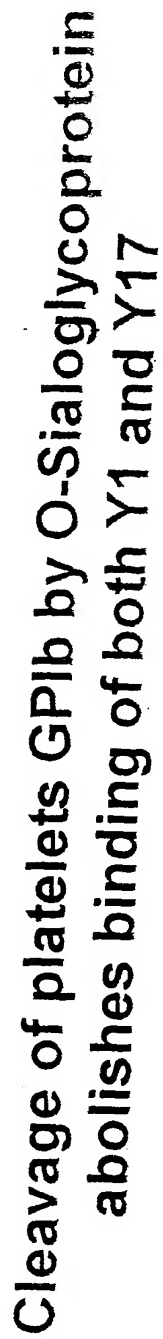




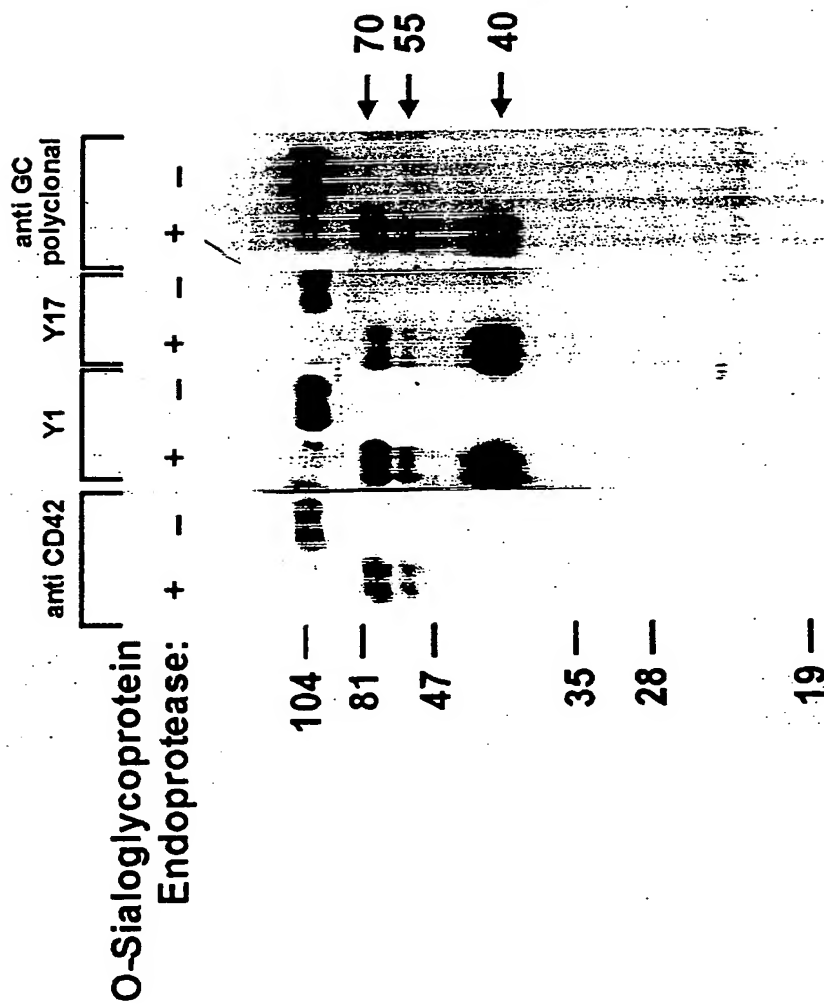
## Characterization of Optimal Determinants for Binding of Y1 to It's Ligands

**FIG. 3**

	Platelets/GC	KG1/RP-HPLC #4
Rec: GP1b 1-340 GP1b 1-480	- -	
Glycanase: N N+O	+ +	+++ +++
Proteases: Mocarhagin O-Sialo Peptidase Ficin Trypsine Elastase	++ (~40kD) ++ (~40kD) - ++ (~40kD) ++ (~40kD)	- - - - ++
Sulfatase (Aryl)		-/+



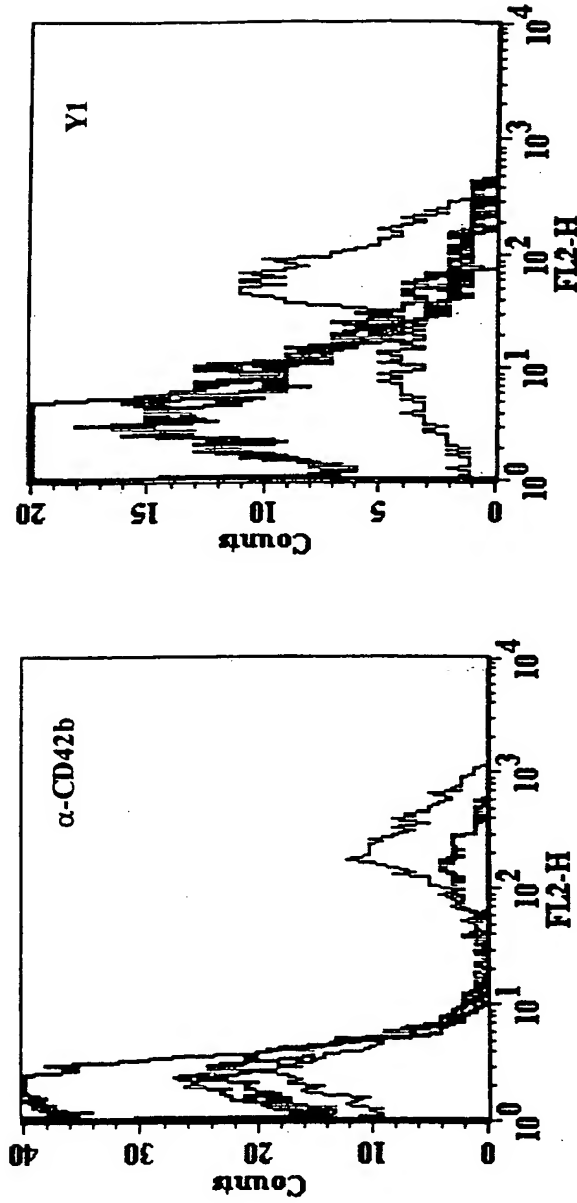
Y1 and Y17 binds similar glycosylated fragments after cleavage by O-Sialoglycoprotein Endoprotease



**FIG. 5**

# Specific GPIIb Proteolysis Abolishes Y1 Binding to Platelets

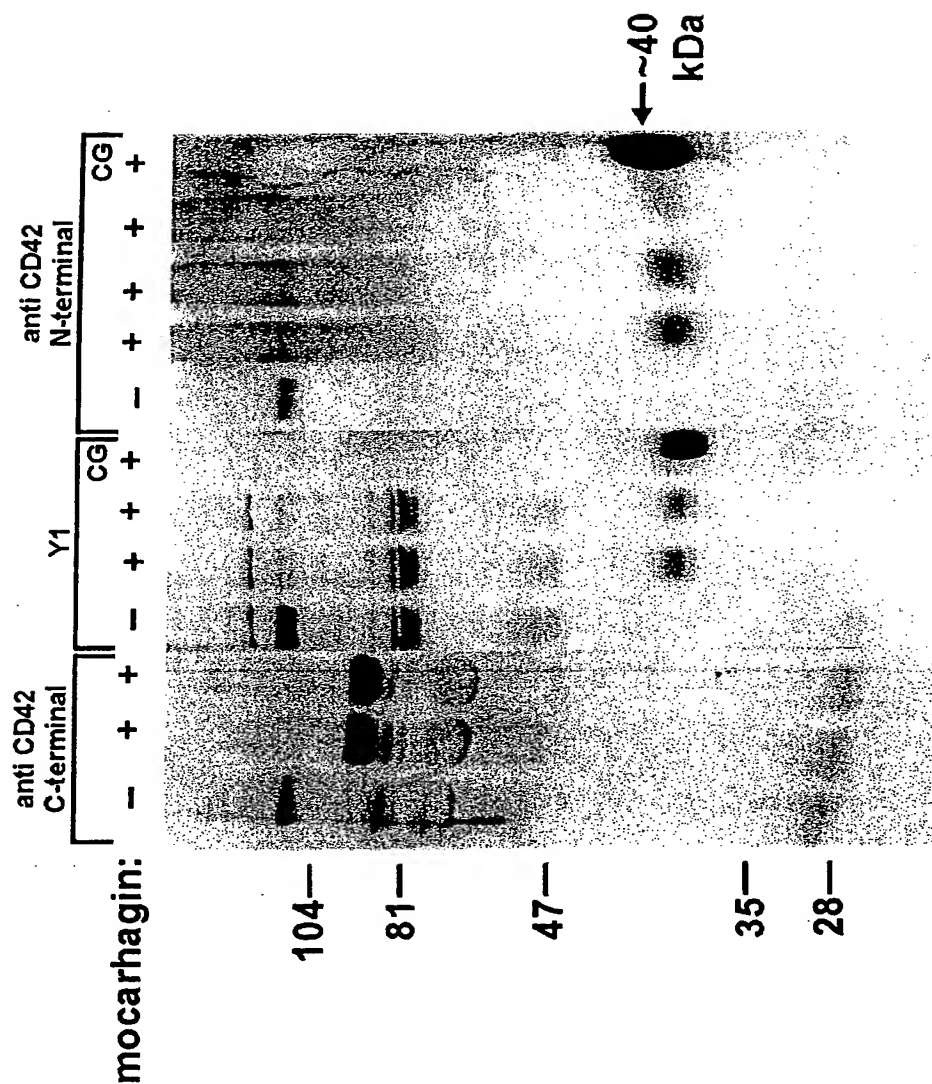
FIG. 6



Key	Name	Parameter	G
	NON-TREATED PLATELETS		
---	O-SIALOGLYCOPROTEIN ENDO. (10 $\mu$ g/ml)		
---	O-SIALOGLYCOPROTEIN ENDO. (50 $\mu$ g/ml)		
---	FICIN (18 $\mu$ g/ml)		

# Y1 binds N-terminal (His-1 - Glu 282) fragment of platelet GPIb after cleavage by mocarhagin

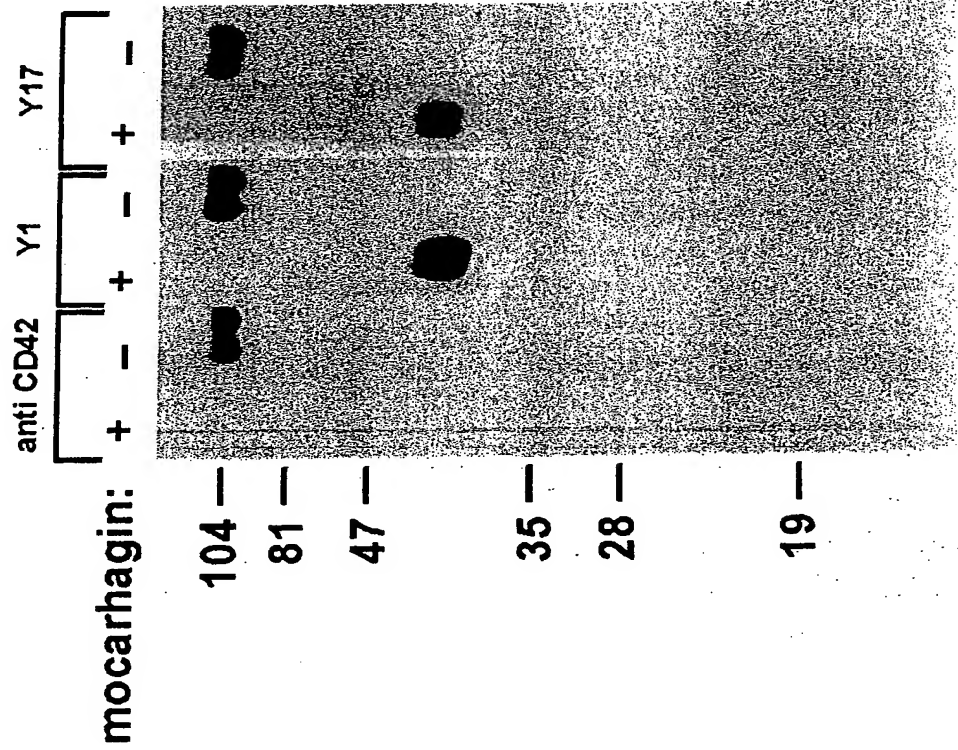
FIG. 7





**FIG. 8**

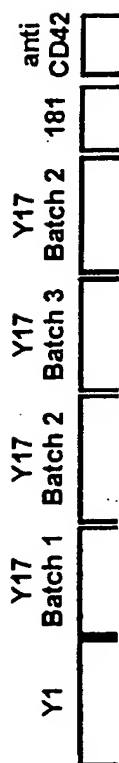
**Binding of Y1 and Y17 to glycolalycin after  
cleavage by mocarhagin**





**FIG. 9**

Binding of Y1 and Y17 to platelets



104—

81—

47—

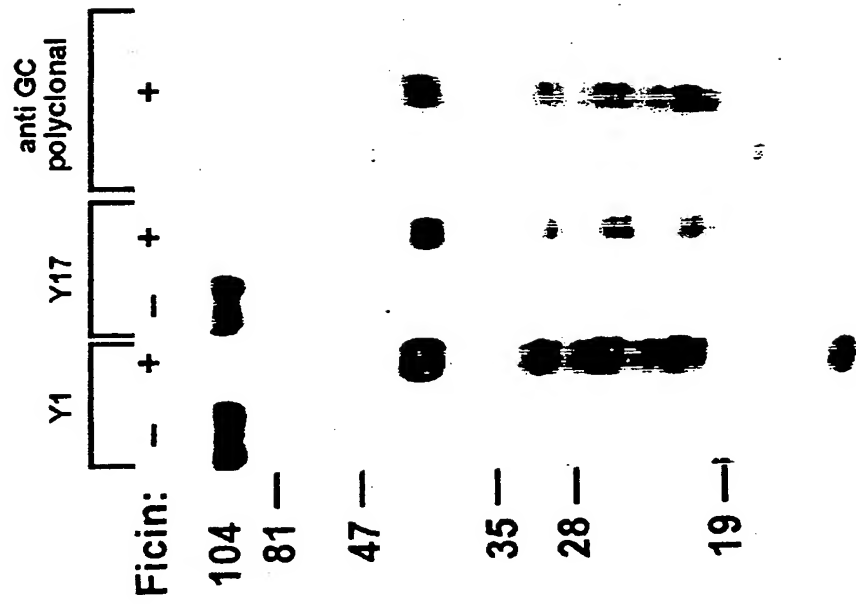
35—

28—

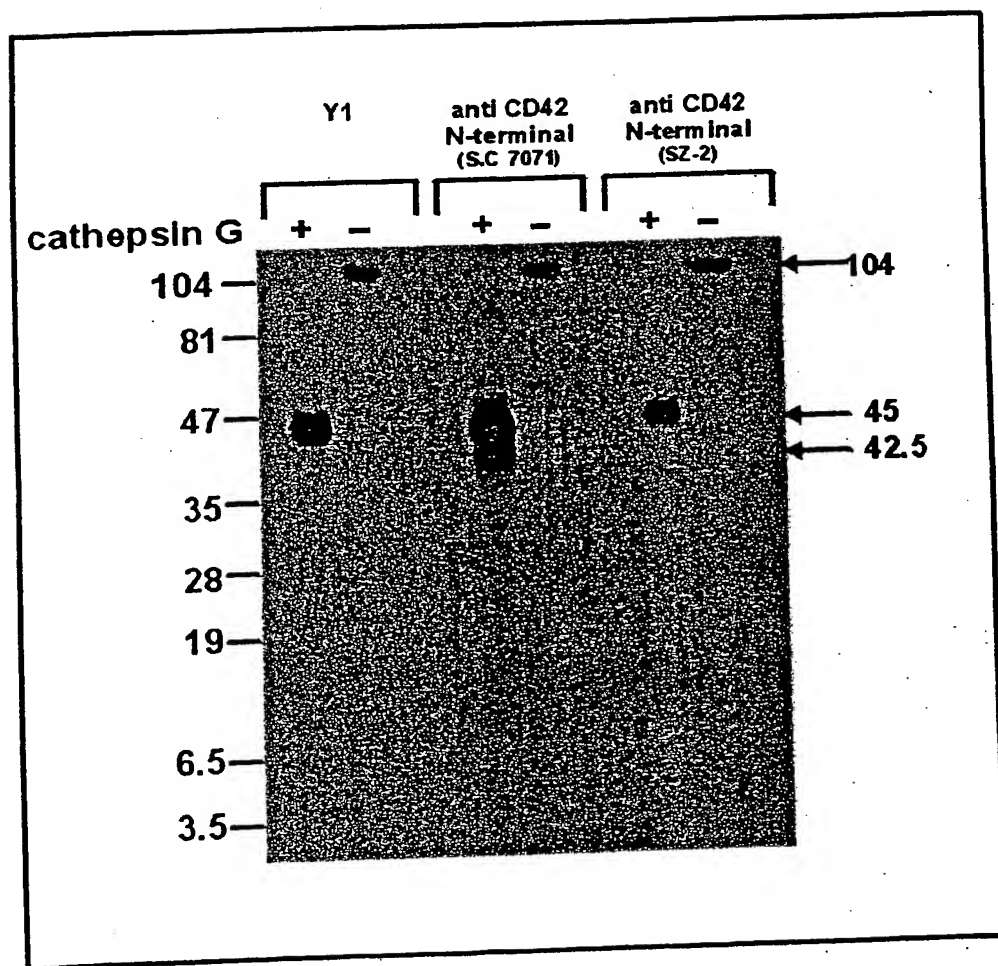


Y1 and Y17 bind glycoprotein similar after cleavage by Ficin

**FIG. 10**

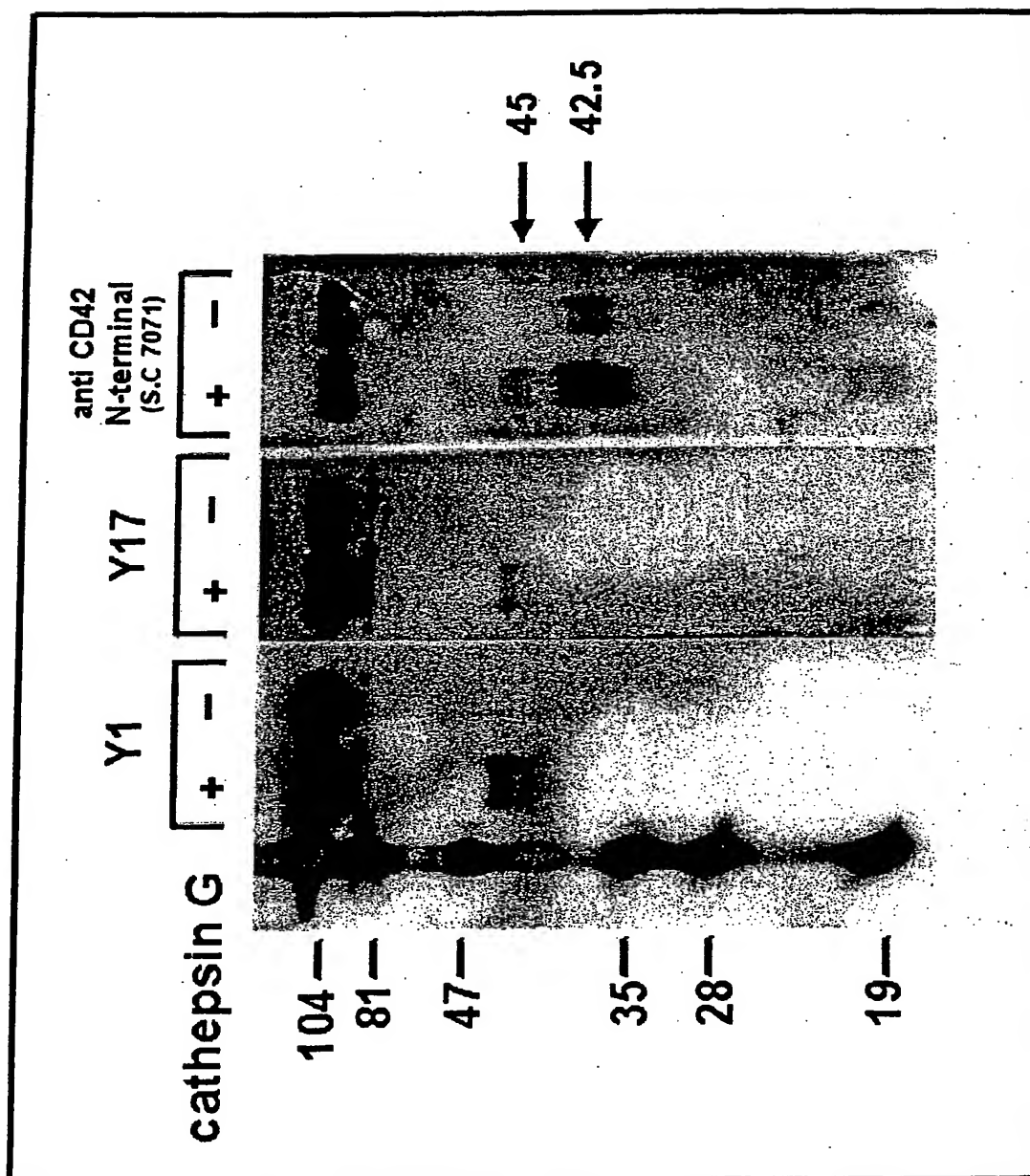


**FIG. 11**



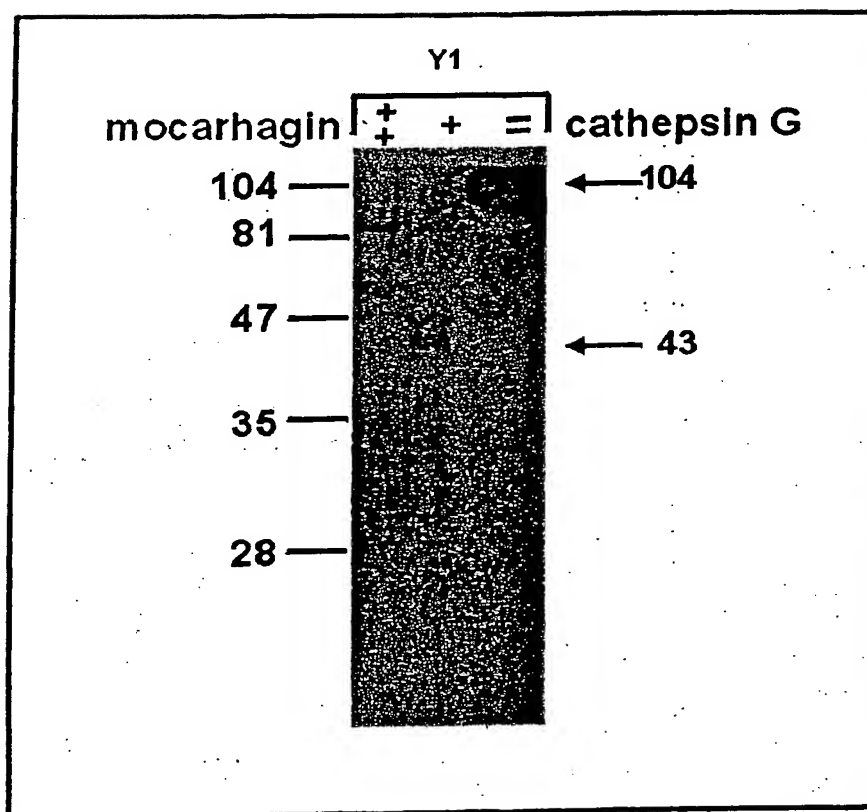
# Y1 and Y17 reacts with larger cathepsin G cleaved platelets GPIIb fragment

FIG. 12





*FIG. 13*



mocarhagin  $\left[ \begin{array}{c} + \\ + \\ + \end{array} \right] \left[ \begin{array}{c} + \\ + \\ + \end{array} \right] \left[ \begin{array}{c} + \\ + \\ + \end{array} \right]$

anti CD42 N-terminal (SZ-2)  $\left[ \begin{array}{c} + \\ + \\ + \end{array} \right] \left[ \begin{array}{c} + \\ + \\ + \end{array} \right] \left[ \begin{array}{c} + \\ + \\ + \end{array} \right]$

anti CD42 C-terminal (Serotec)  $\left[ \begin{array}{c} + \\ + \\ + \end{array} \right] \left[ \begin{array}{c} + \\ + \\ + \end{array} \right] \left[ \begin{array}{c} + \\ + \\ + \end{array} \right]$

anti CD42 N-terminal (S.C 7071)  $\left[ \begin{array}{c} + \\ + \\ + \end{array} \right] \left[ \begin{array}{c} + \\ + \\ + \end{array} \right] \left[ \begin{array}{c} + \\ + \\ + \end{array} \right]$

Y1  $\left[ \begin{array}{c} + \\ + \\ + \end{array} \right] \left[ \begin{array}{c} + \\ + \\ + \end{array} \right] \left[ \begin{array}{c} + \\ + \\ + \end{array} \right]$

cathepsin G  $\left[ \begin{array}{c} + \\ + \\ + \end{array} \right] \left[ \begin{array}{c} + \\ + \\ + \end{array} \right] \left[ \begin{array}{c} + \\ + \\ + \end{array} \right]$

90  $\rightarrow$

43  $\rightarrow$

42.5  $\rightarrow$

104 —

81 —

47 —

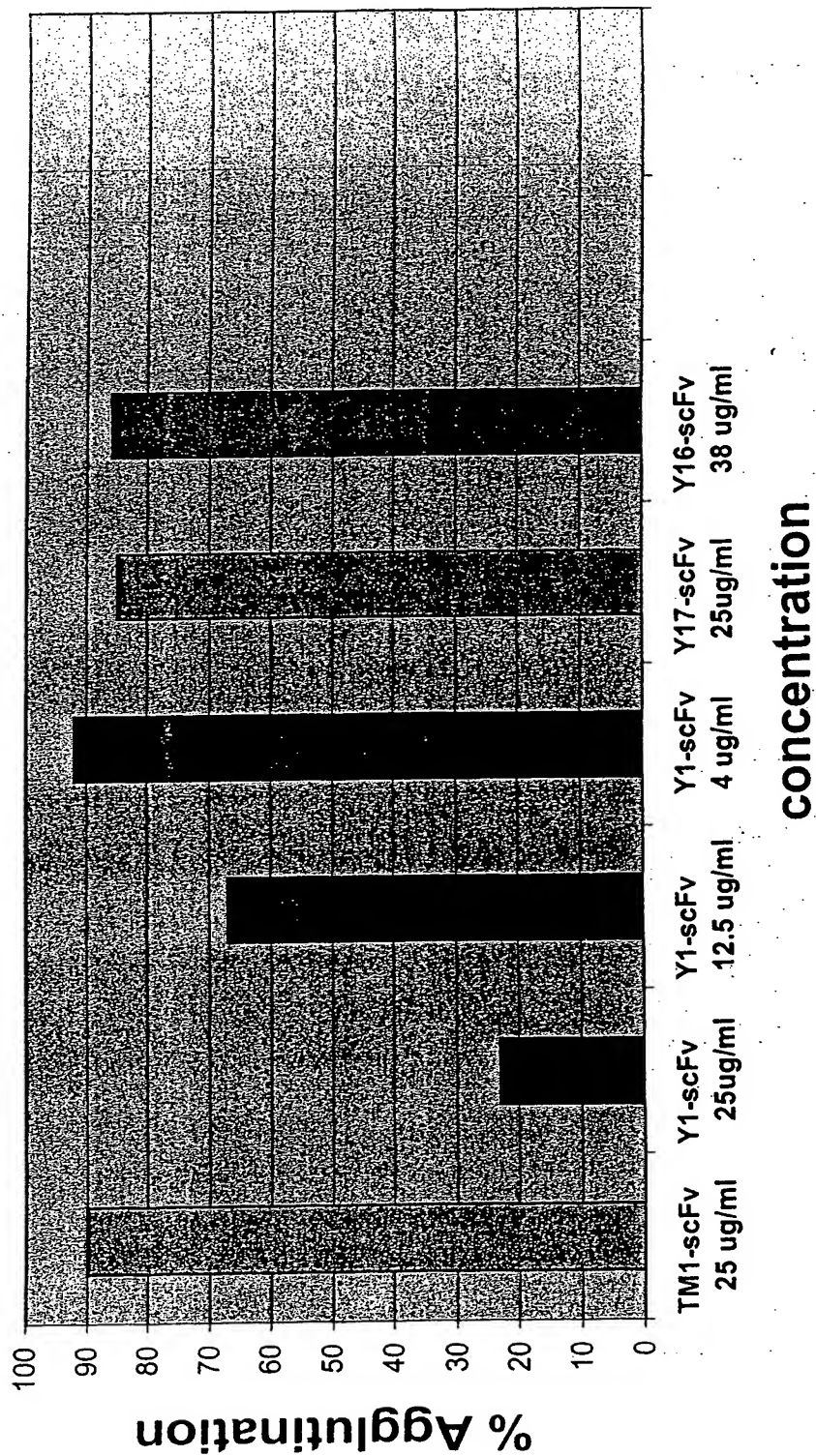
35 —

28 —

19 —

**FIG. 15**

**Influence of Y1-scFv on platelets agglutination in washed platelets**





**FIG. 16**

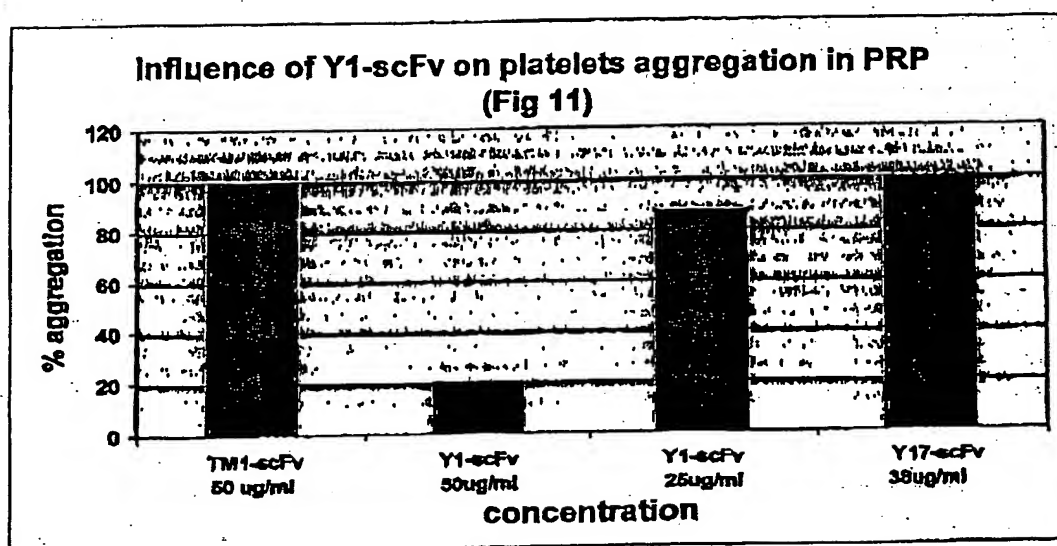
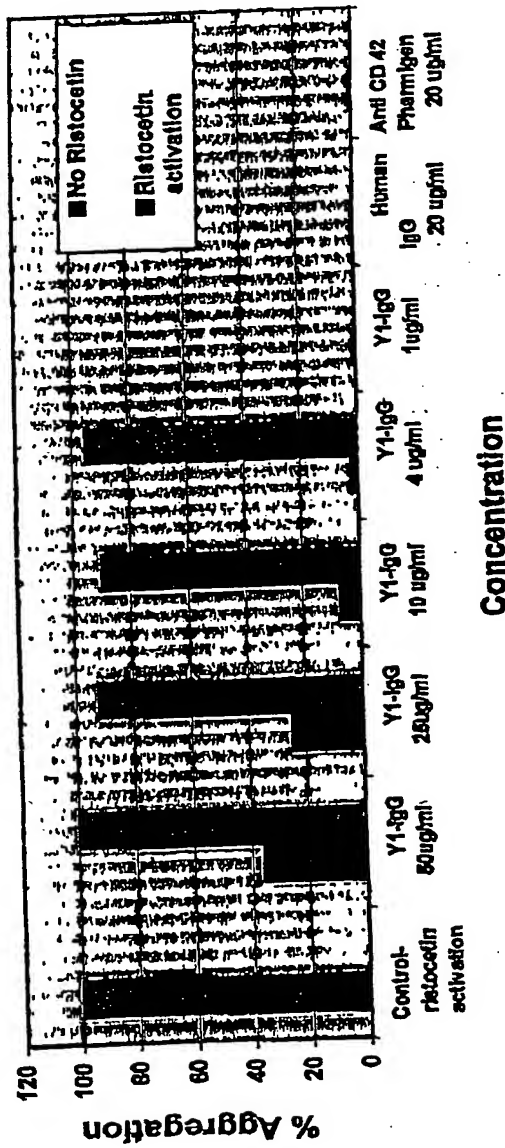






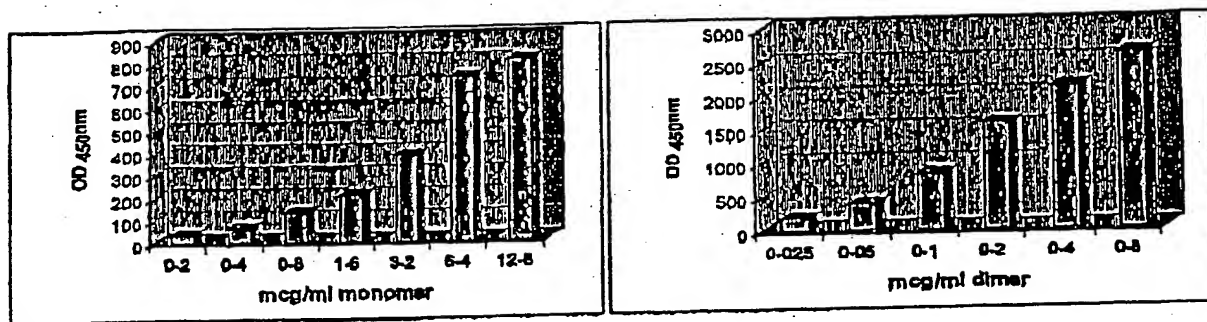
FIG. 18

Induction of platelet aggregation by Y1-IgG in PRP





**FIG. 19**



# Specificity of Binding of Y1 and $\alpha$ -CD42 (N1-19) to their Ligands

**FIG. 20**

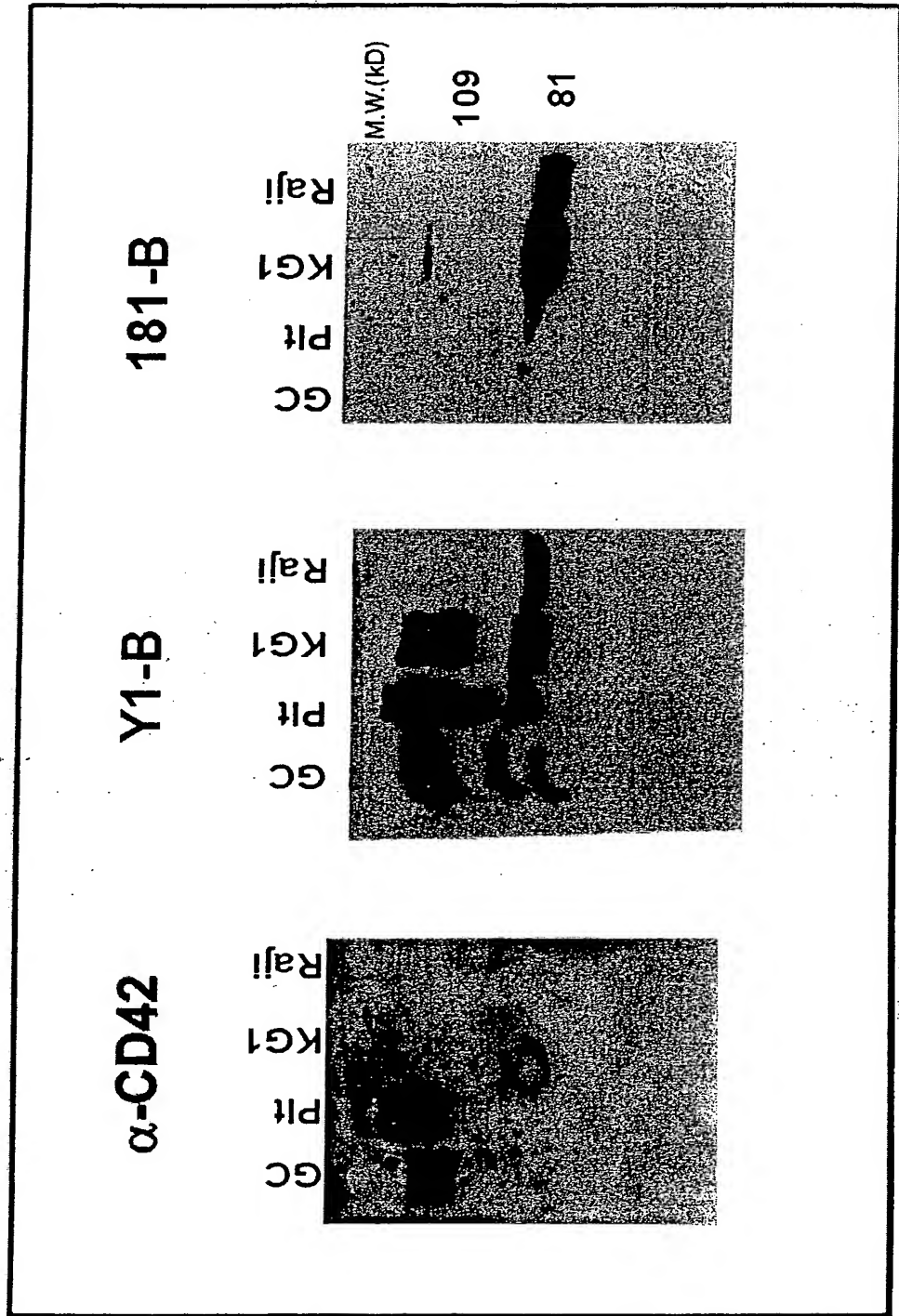
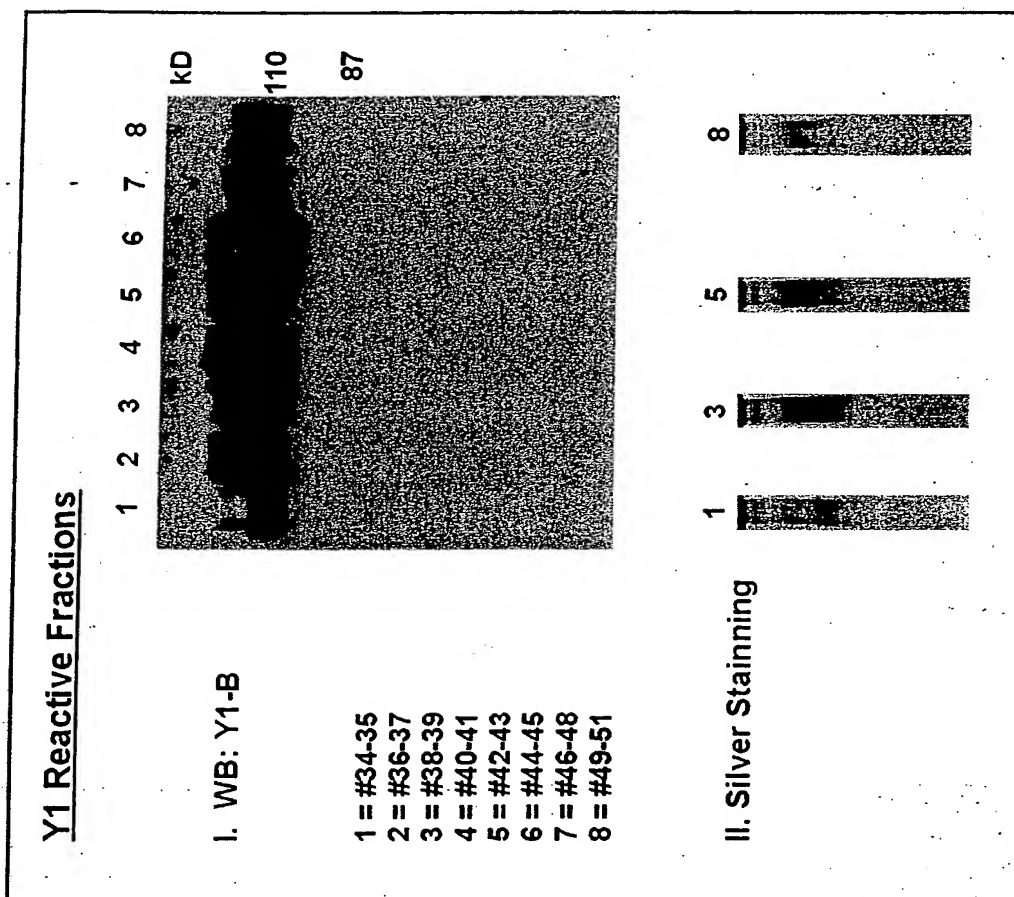


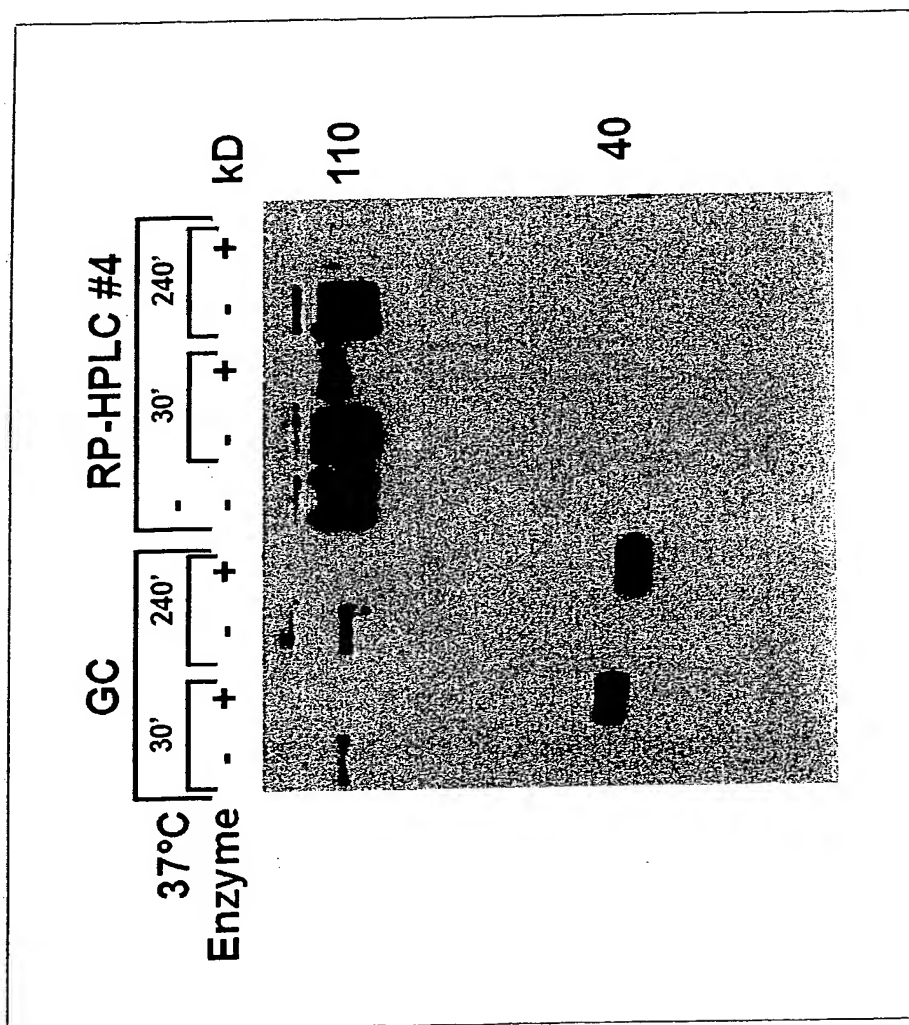
FIG. 21

# Y1-Ligand from KG1 membranes following Immuno-Precipitation with Y1: Purification on RP-HPLC



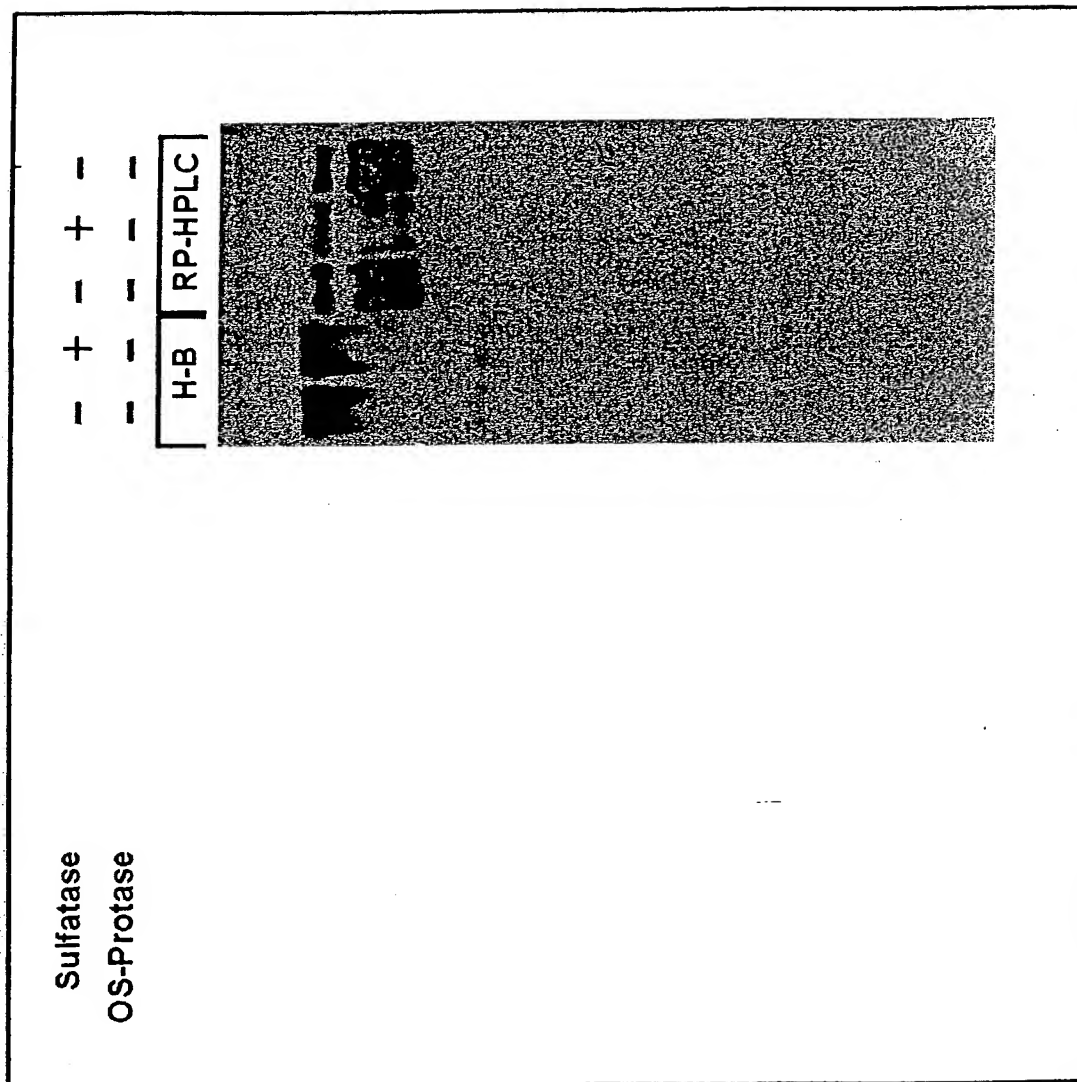
# Effect of O-Sialo-Glycoprotein Endopeptidase on Y1 Binding

FIG. 22



# Effect of Aryl-Sulfatase on Binding of Y1: RP-HPLC(KG1) & H-B(Heparin-BSA)

**FIG. 23**



# Specificity of Y1 Binding: Analysis by Immune Precipitation with Y1 and anti-PSGL-1

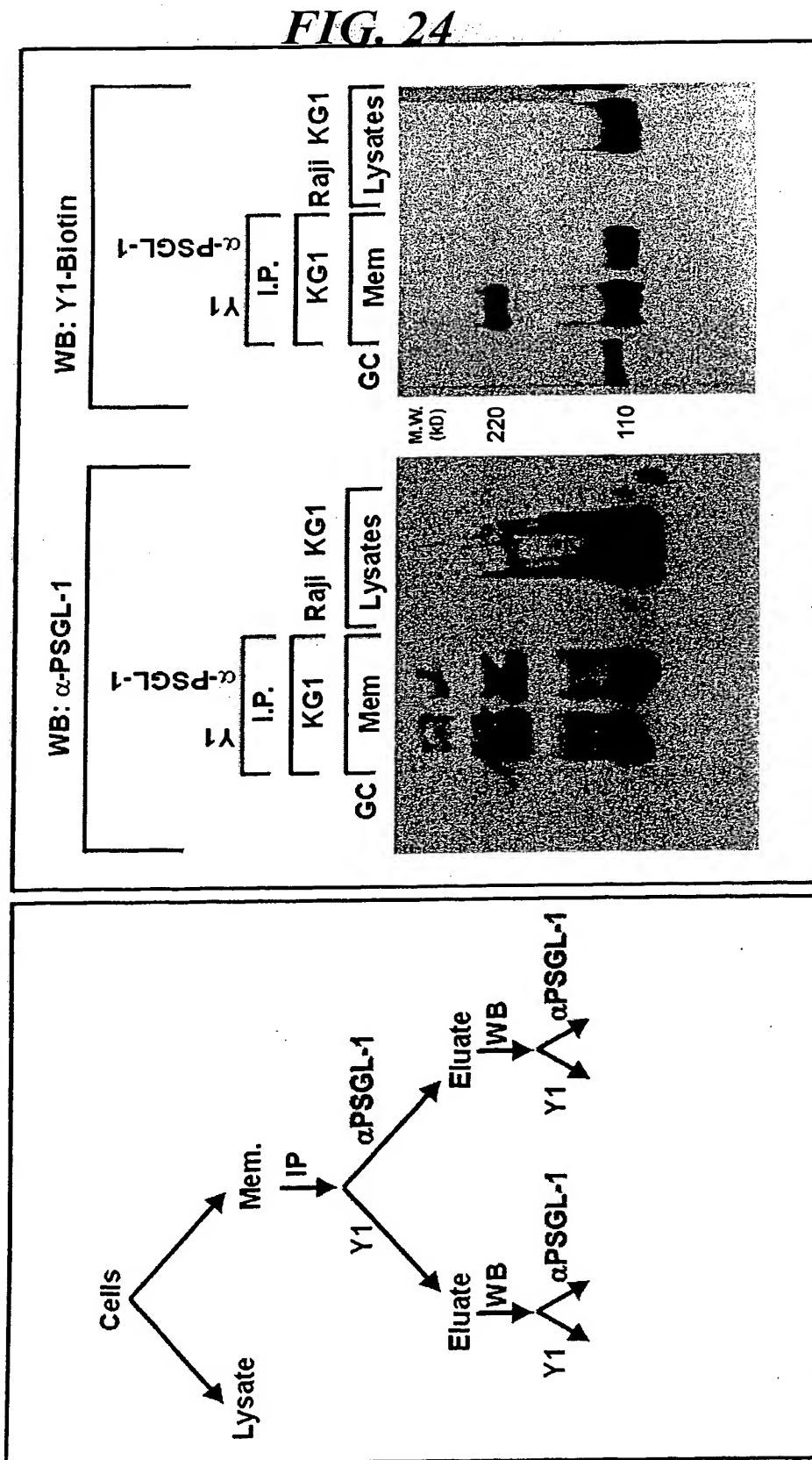




FIG. 25

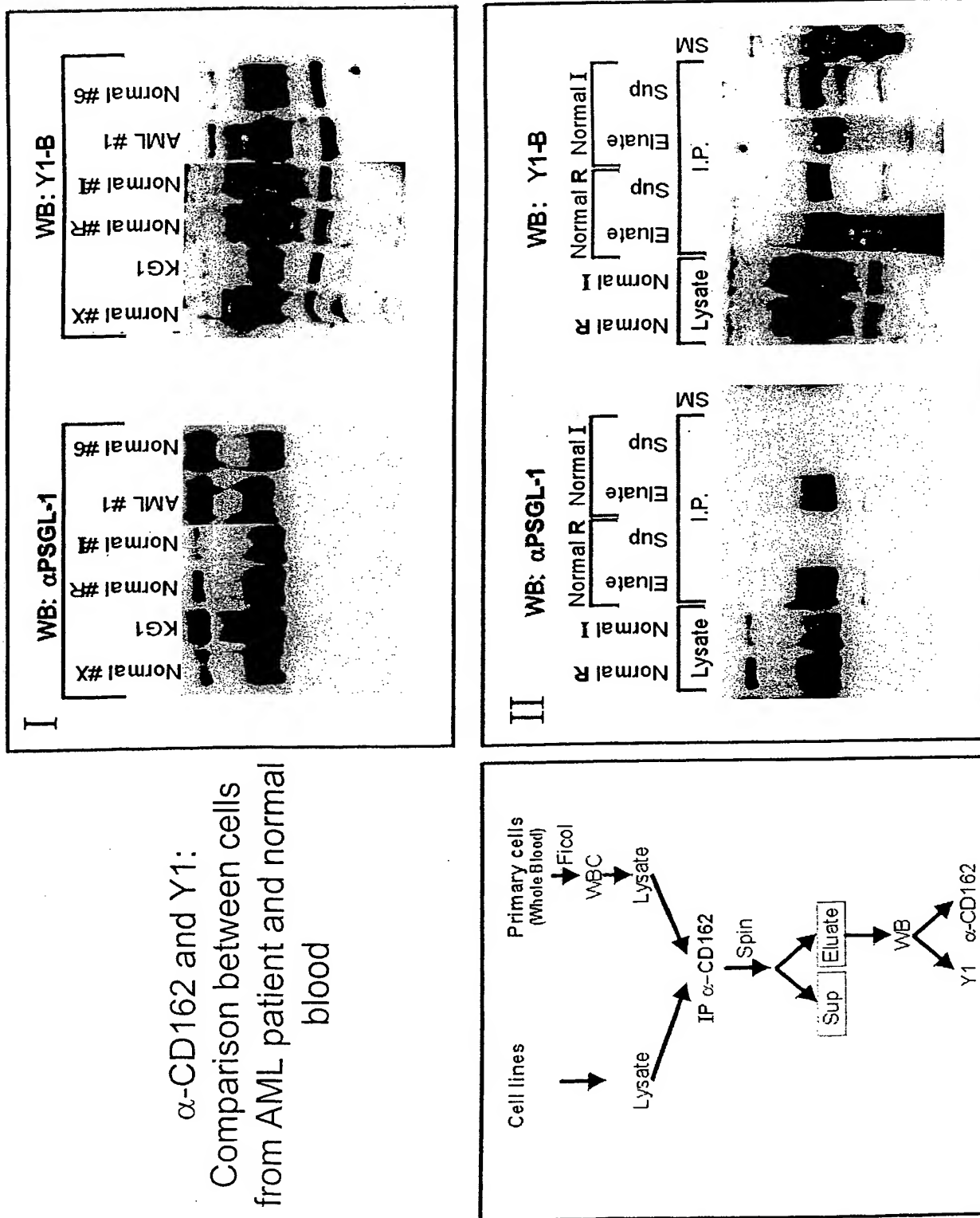
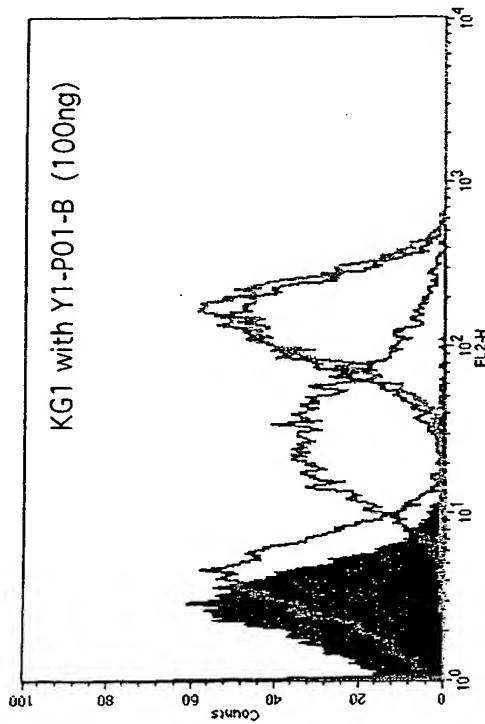
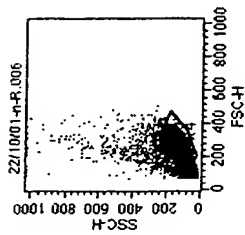
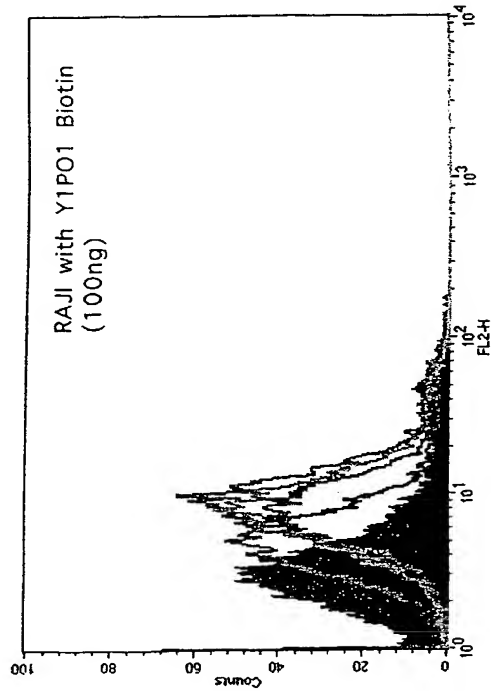
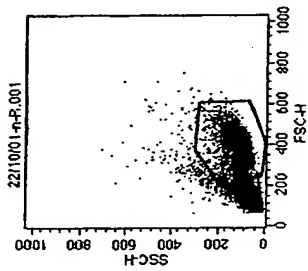


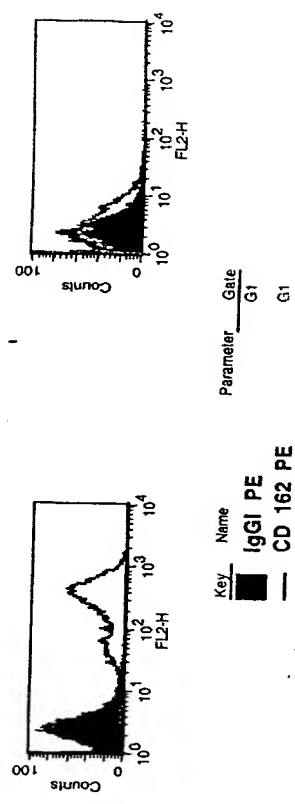


FIG. 26



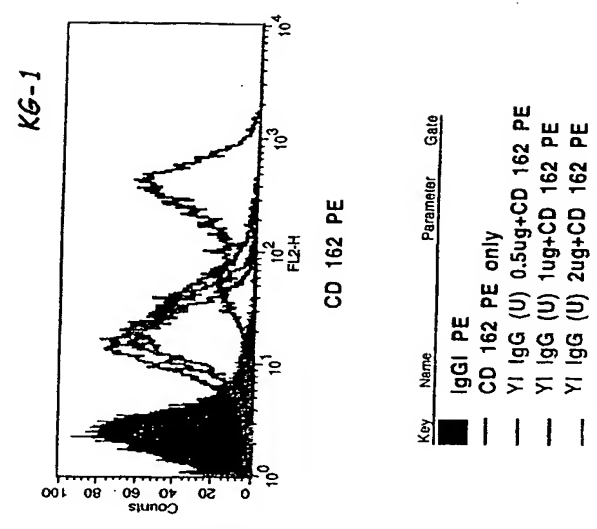
Key	Name	Parameter	Gate
1	2210101-n-R.006	N01-B	
2	2210101-n-R.007	P01-B	
3	2210101-n-R.008	+KPL1	
4	2210101-n-R.009	+PL1	
5	2210101-n-R.010	+PL2	

# Specificity of Y1 Binding: Analysis by FACS



- Binding of  
α PSGL1  
(αCD162/KPL1);  
competition  
with Y1-IgG

FIG. 27





**FIG. 28**

# Specificity of Y1 Binding: Analysis by FACS

- Binding of  
Y1-IgG;  
competition  
with  $\alpha$ PSGL-1  
(CD162 /KPL1)

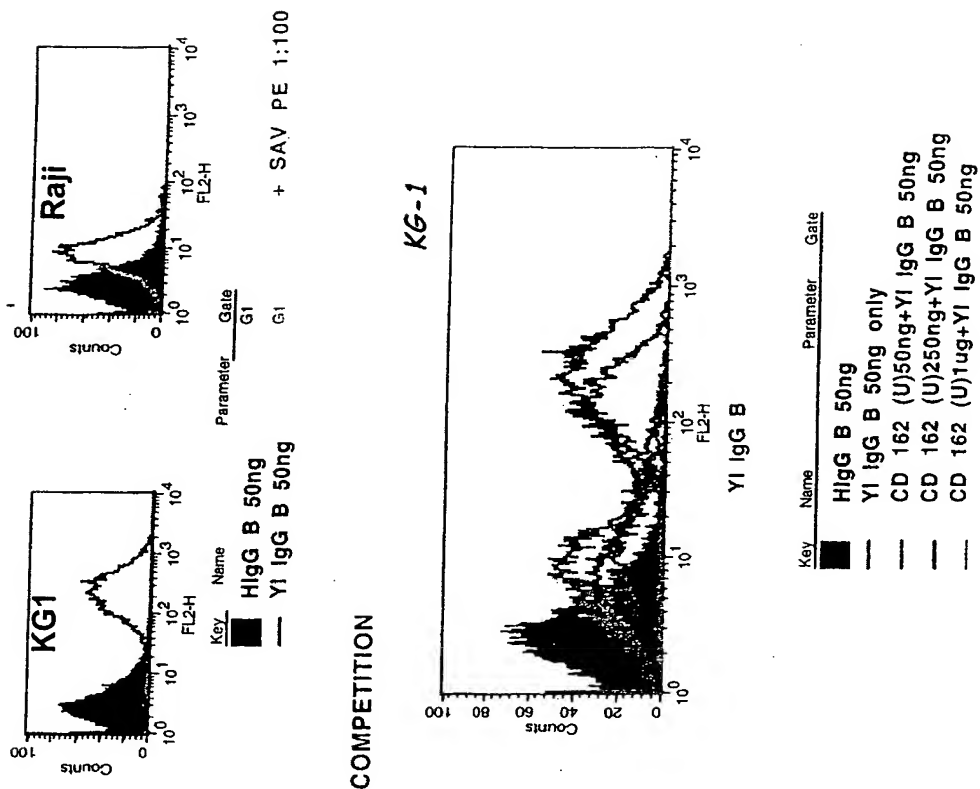
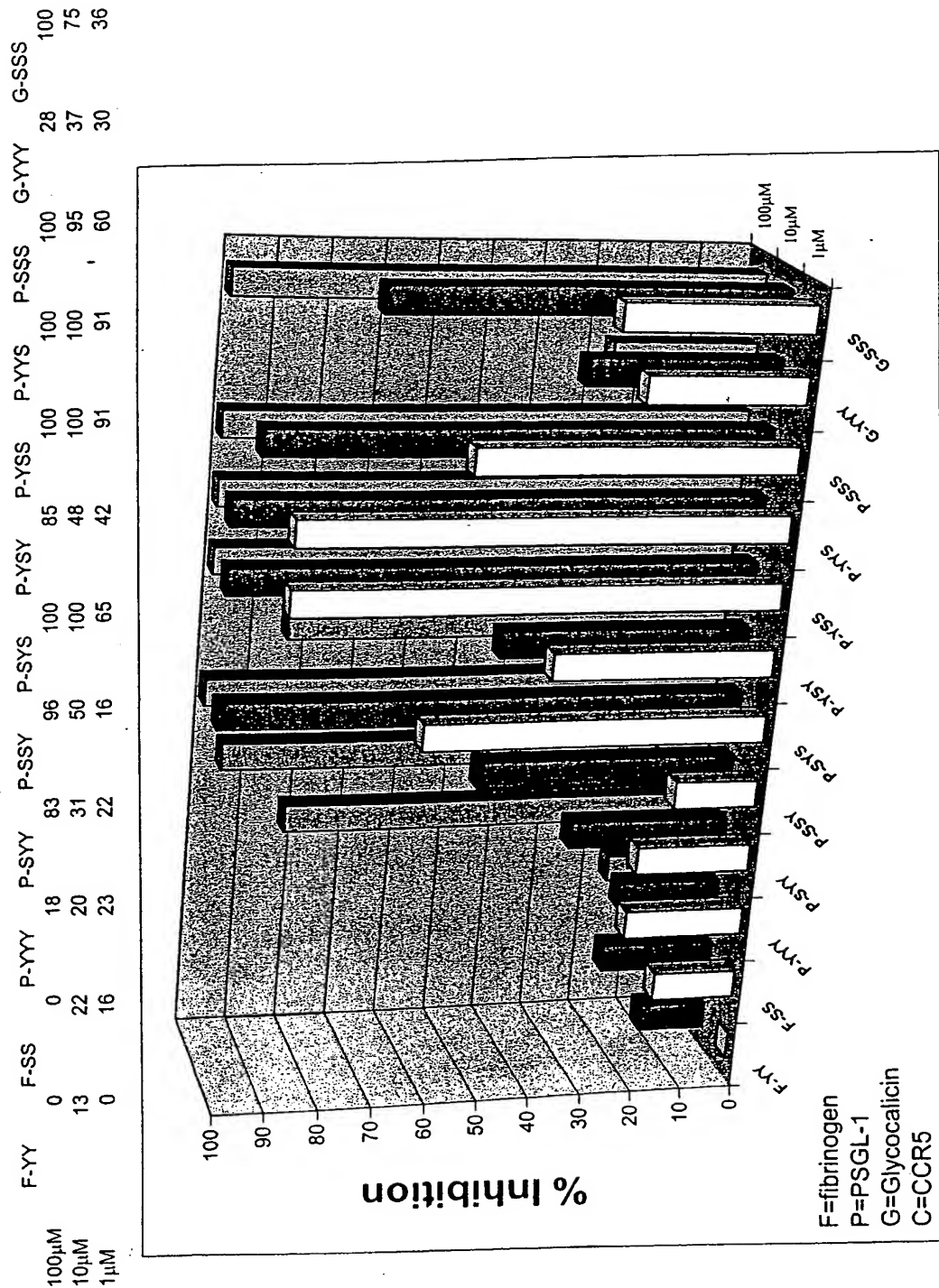
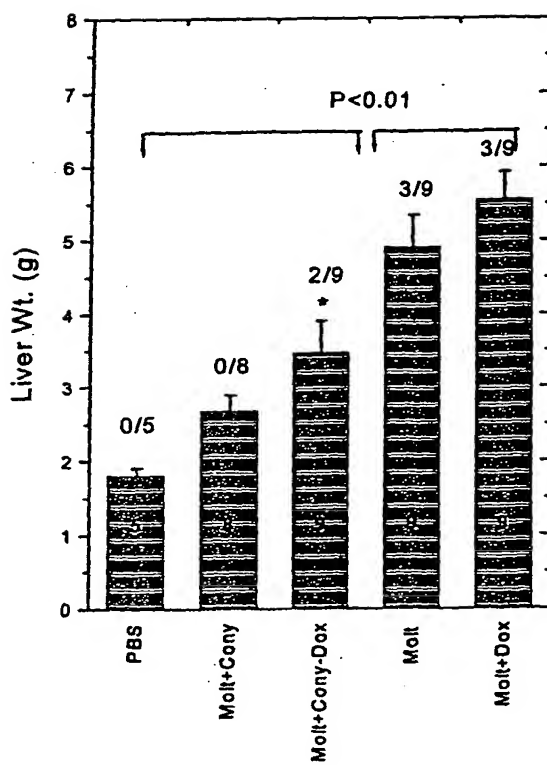


FIG. 29





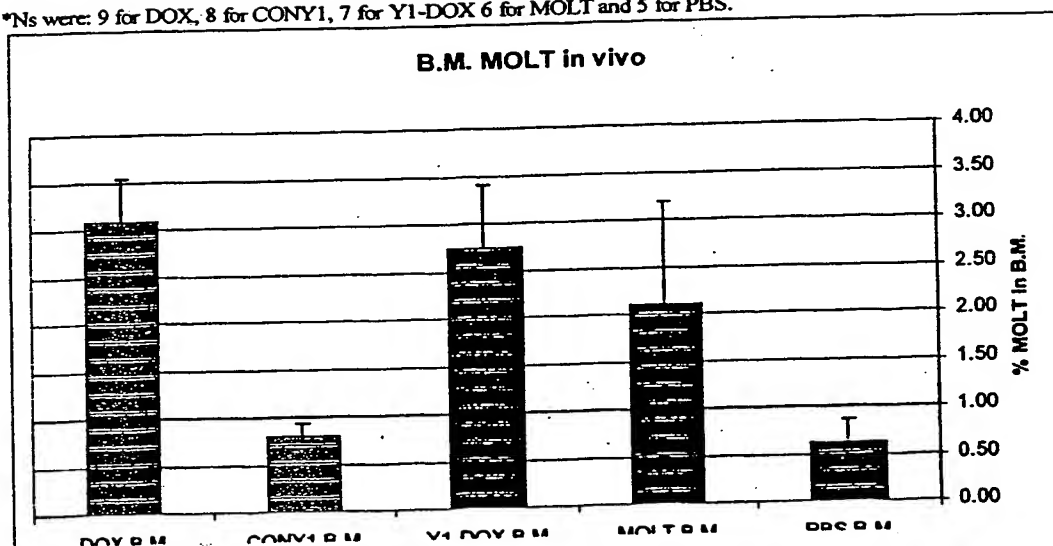
**FIG. 30**





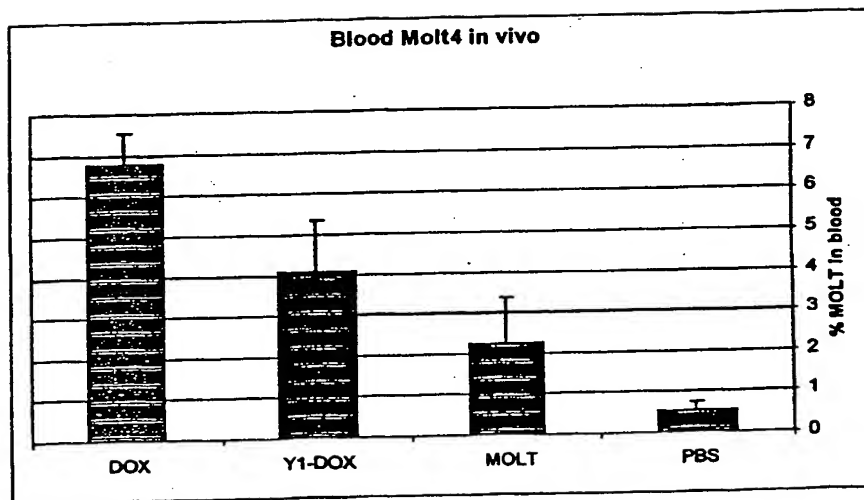
**FIG. 31**

\*Ns were: 9 for DOX, 8 for CONY1, 7 for Y1-DOX 6 for MOLT and 5 for PBS.





**FIG. 32**

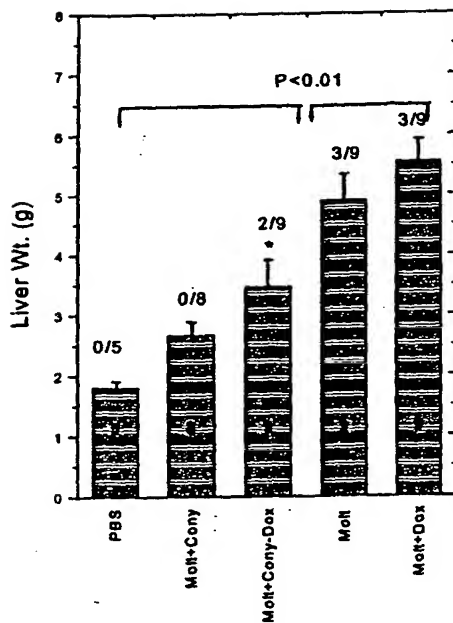


\*\*Ns were: 4 for DOX, 2 for Y1-DOX, 3 for MOLT and 3 for PBS.



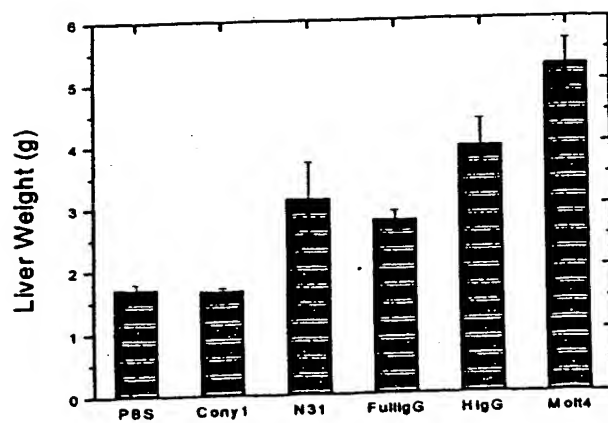


**FIG. 33**



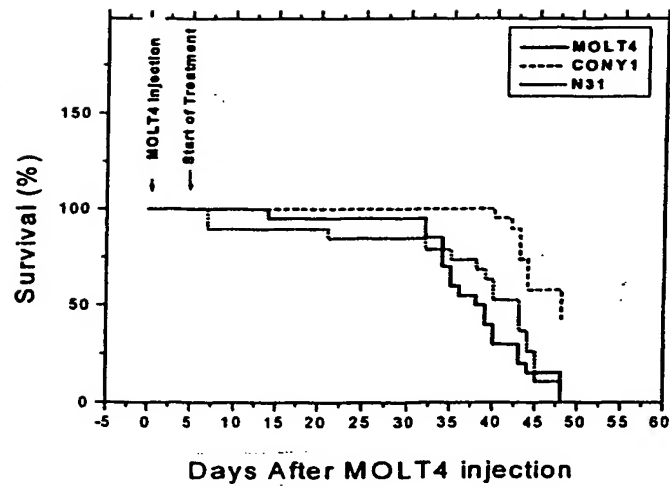


*FIG. 34*





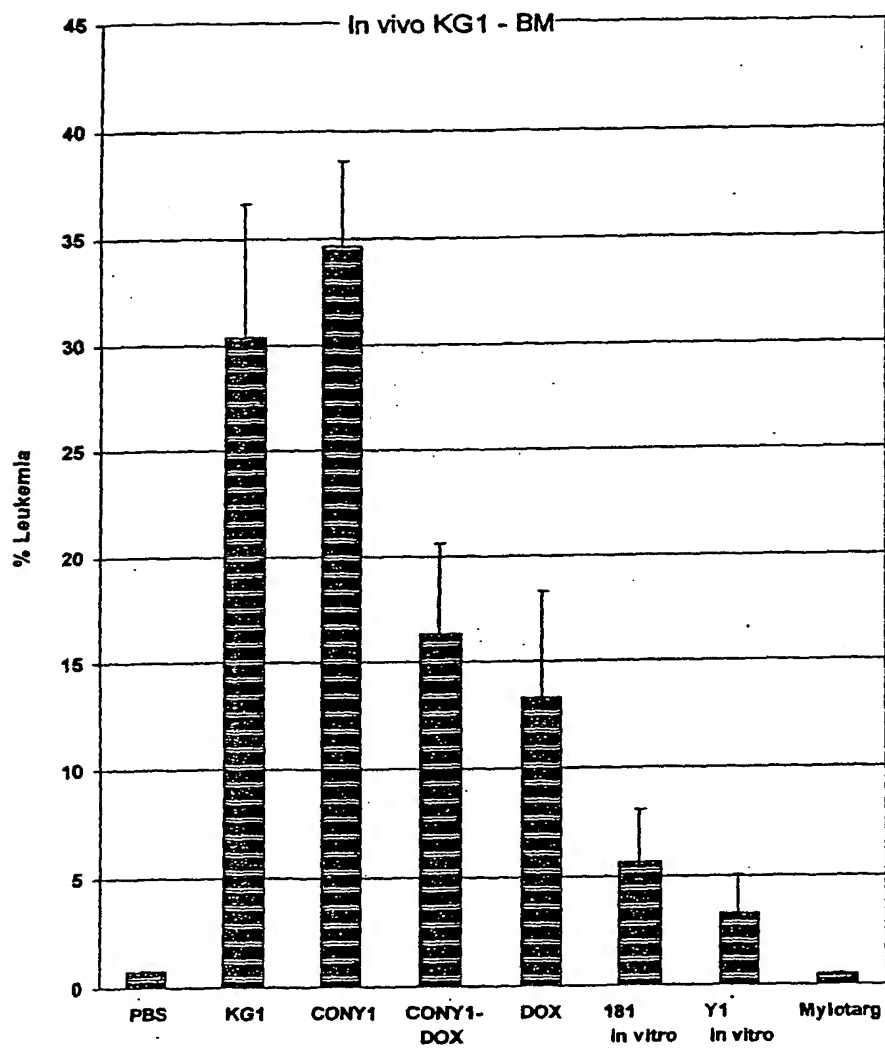
**FIG. 35**





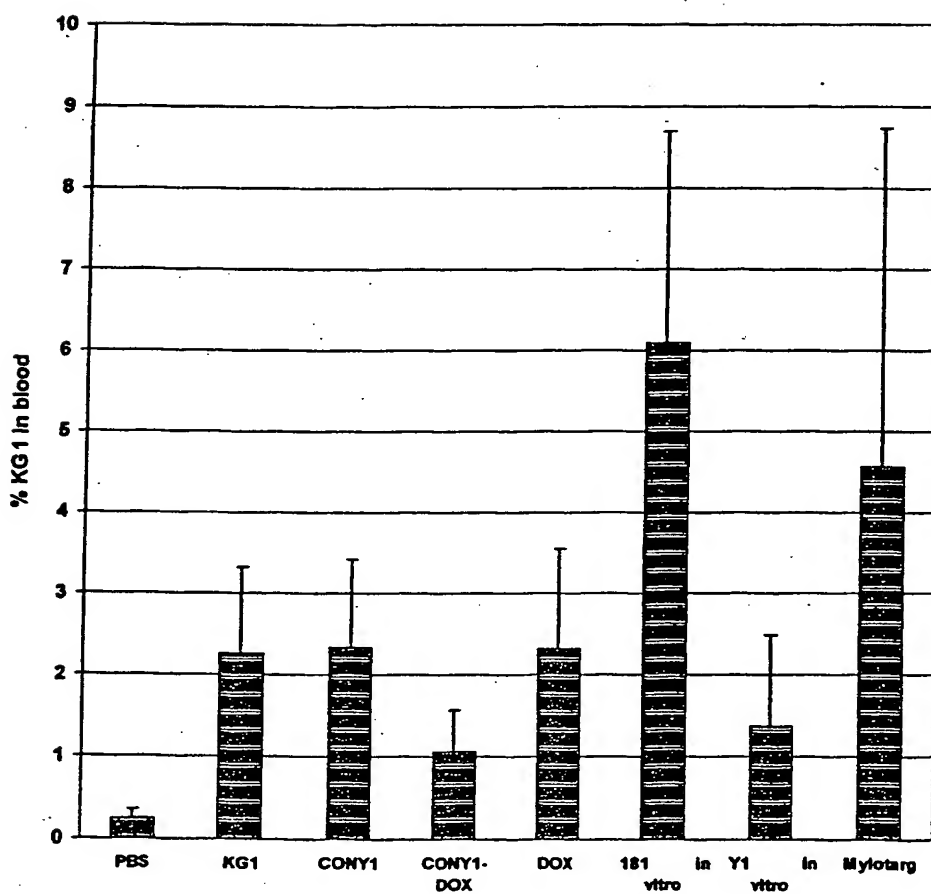
**FIG. 36**

\*\*\*Ns were: 8 for PBS, 9 for KG1, 8 for CONY1, 11 for CONY1-DOX, 9 for DOX, 8 for 181 in vitro, 9 for Y1 in vitro and 9 for Mylotarg.



**FIG. 37**

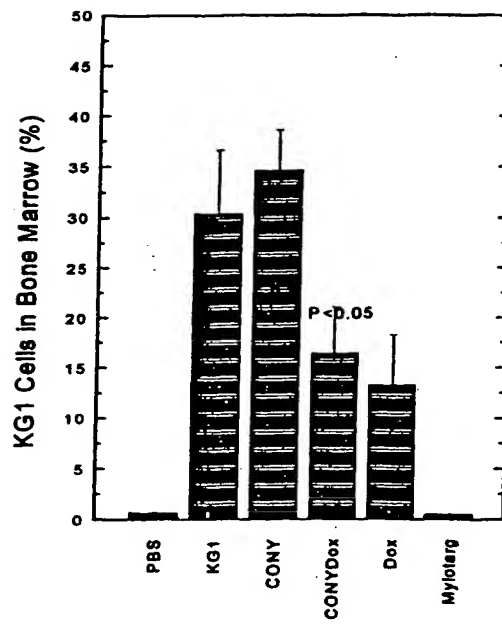
**In vivo KG1 - Blood**



\*\*\*\*Ns were: 8 for PBS, 9 for KG1, 8 for CONY1, 9 for CONY1-DOX, 11 for DOX (including one mice injected with 5mg/kg DOX), 7 for 181 in vitro, 8 for Y1 in vitro and 7 for Mylotarg.

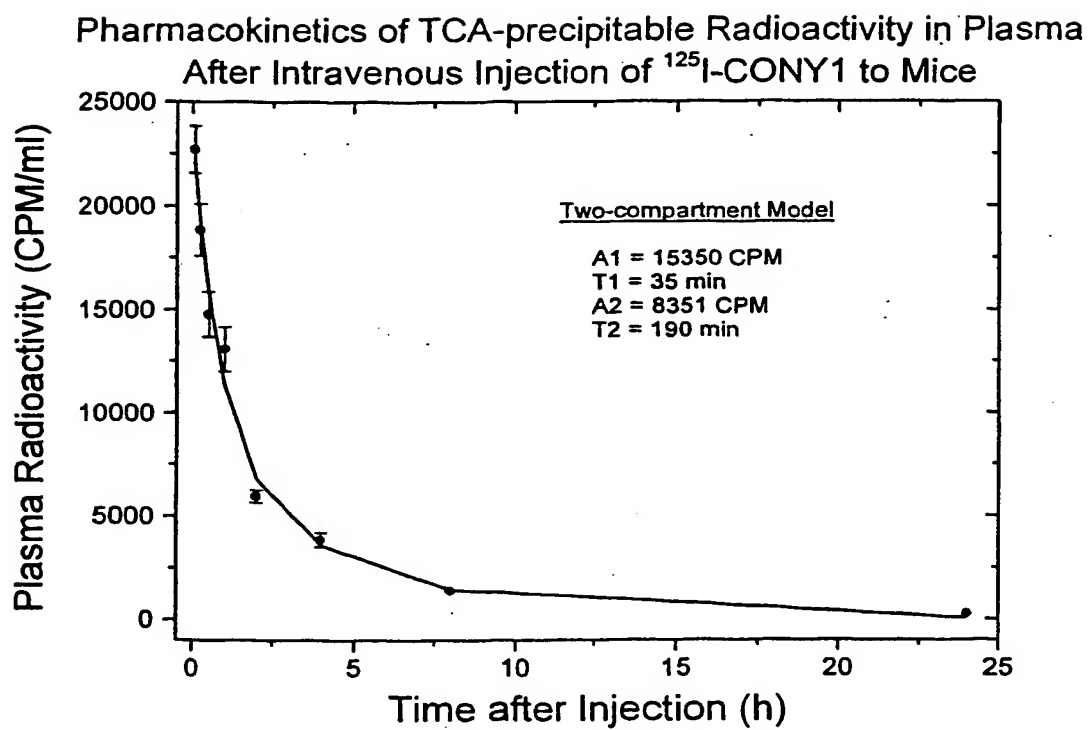


**FIG. 38**



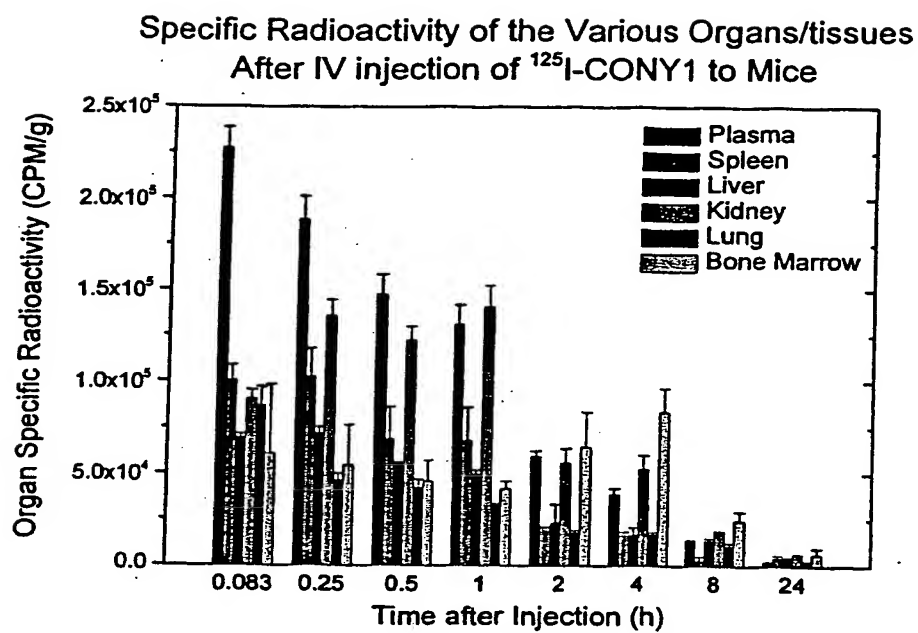


**FIG. 39**





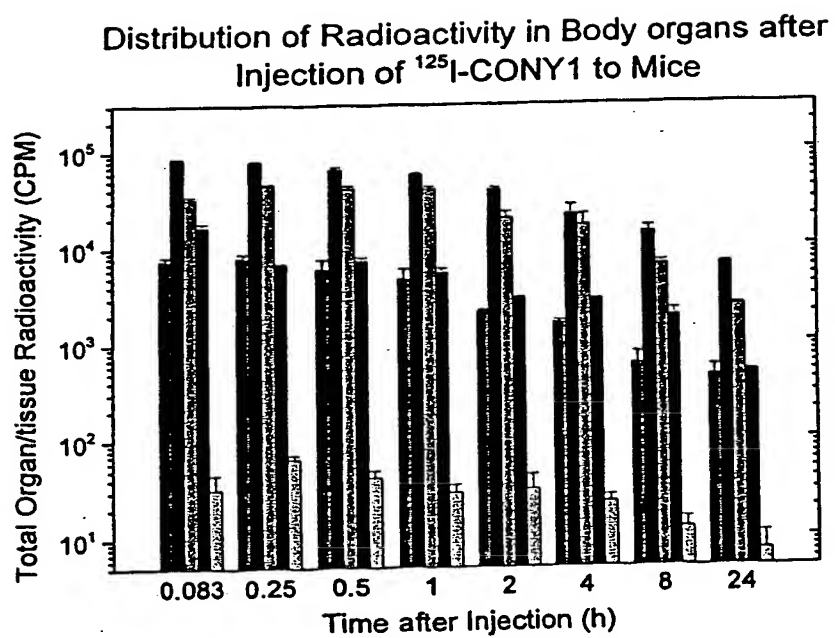
**FIG. 40**





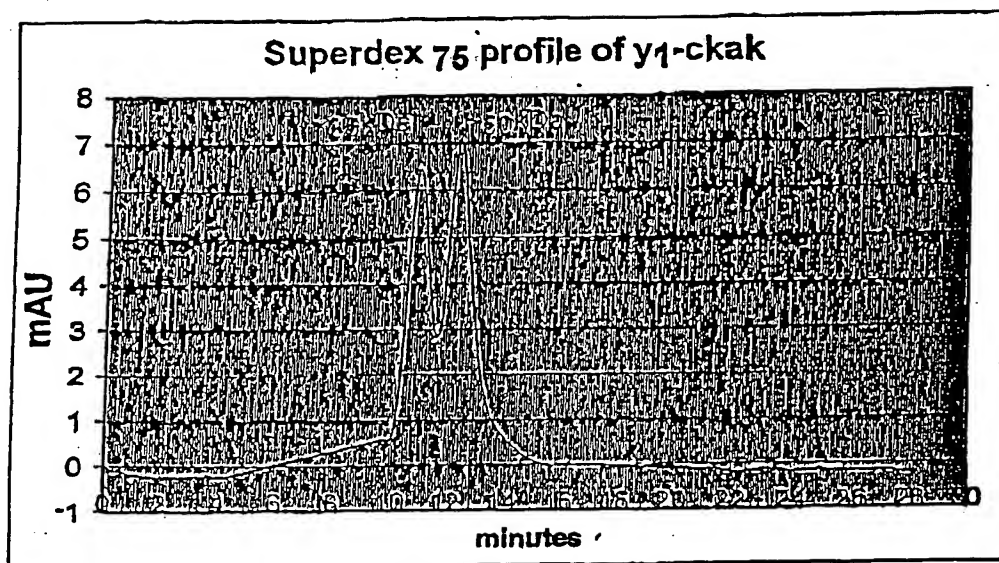


**FIG. 41**

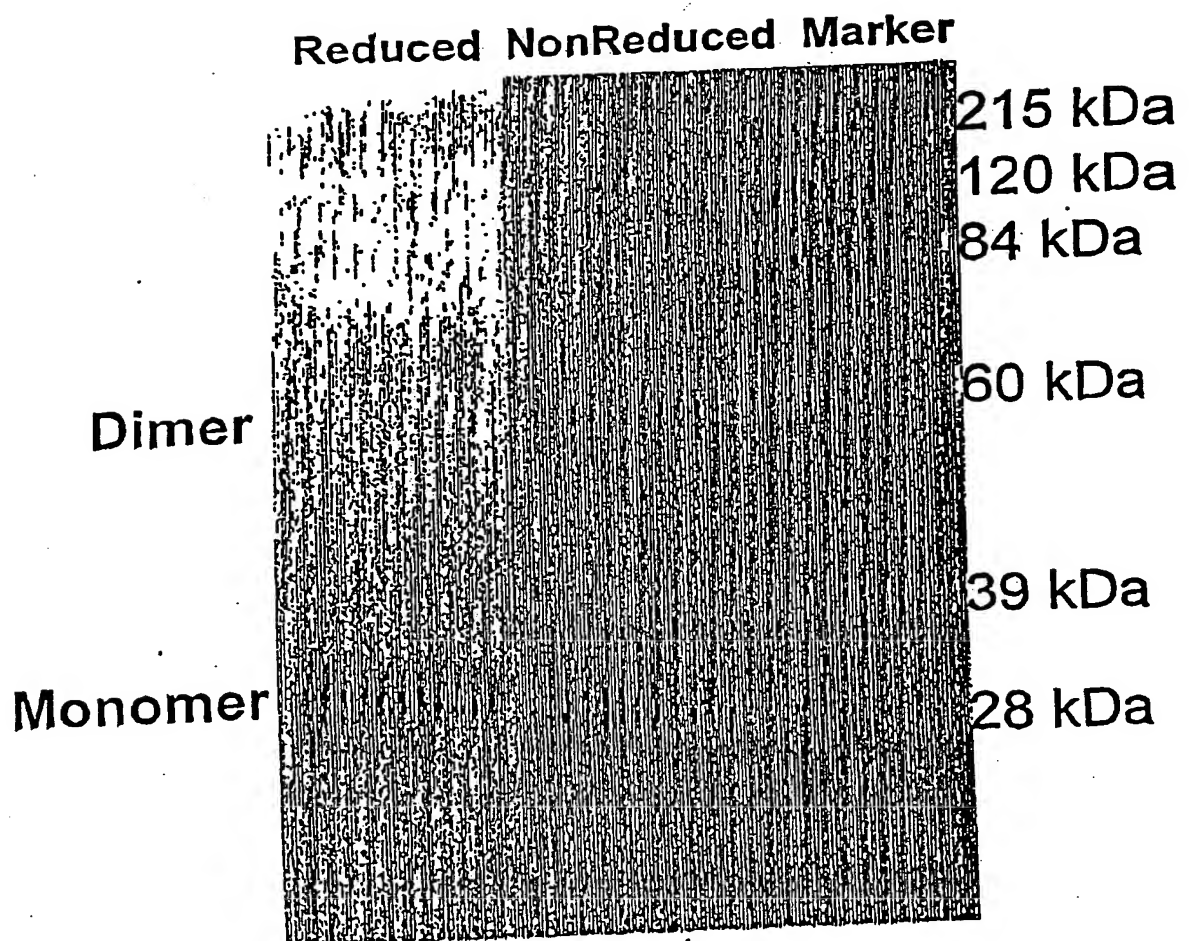




**FIG. 42**

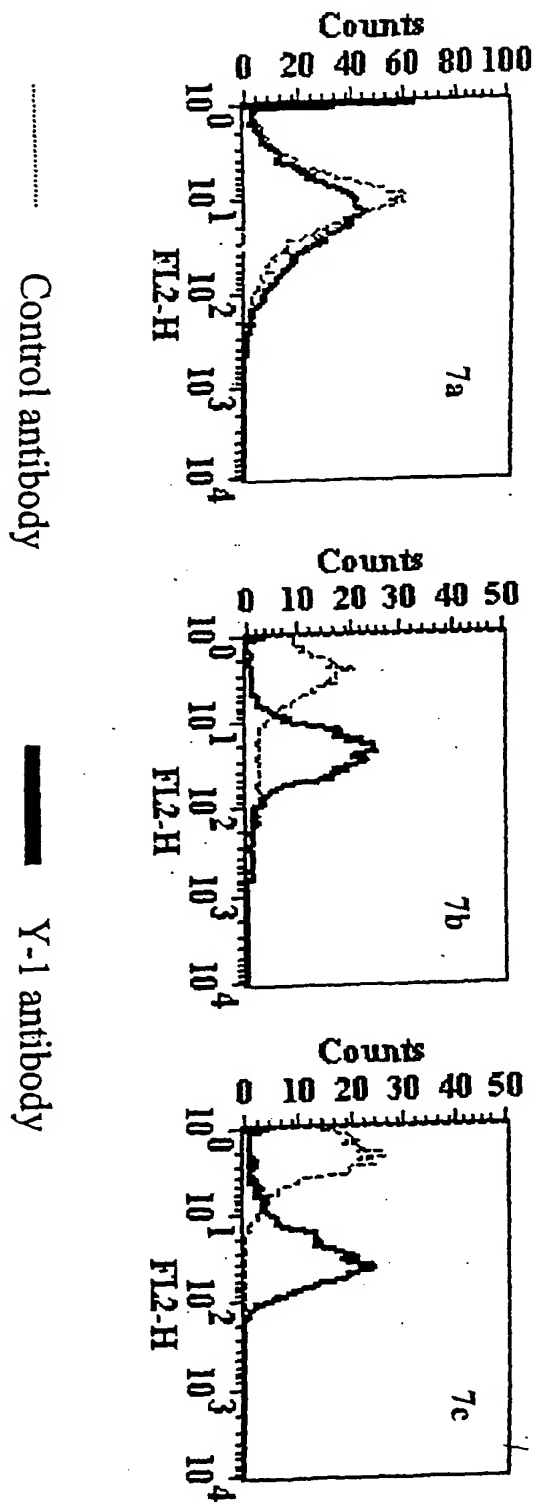


*FIG. 43*





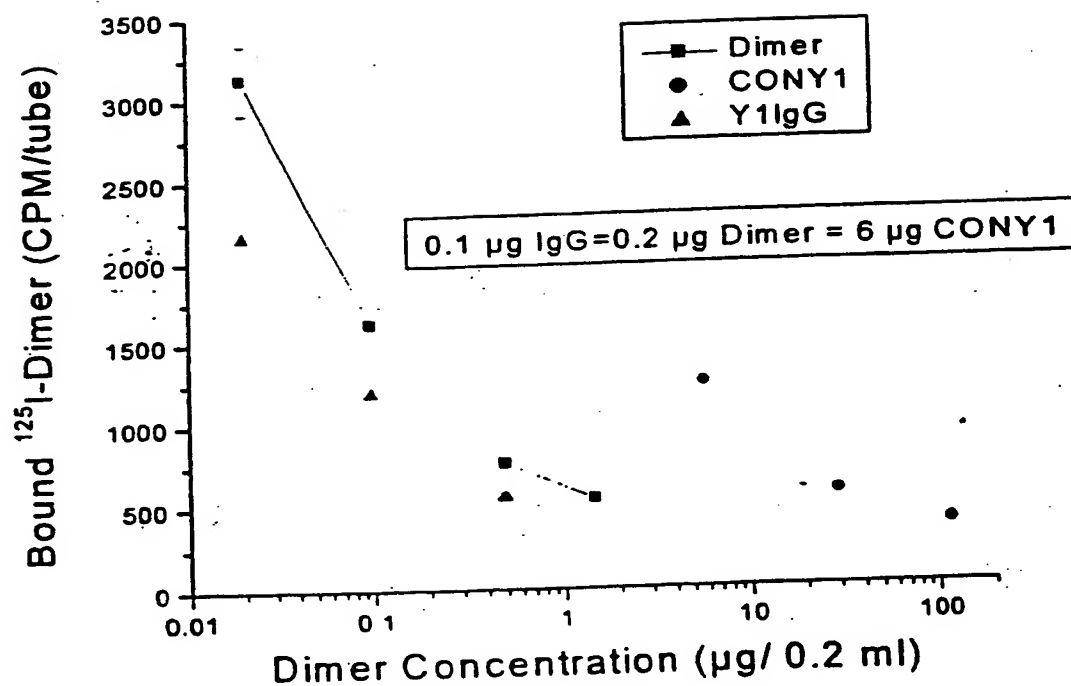
**FIG. 44**







**FIG. 46**





**FIG. 47**

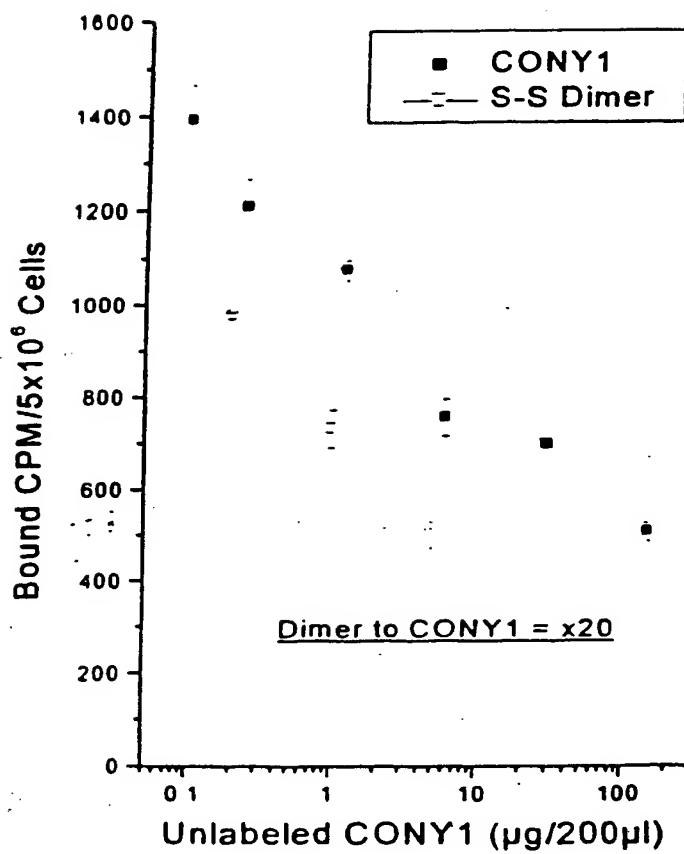




FIG. 48A: The ORF and Amino Acid Sequence of Y1-HC

SEQ ID NO: 205 (nucleic acid sequence): SEQ ID NO: 206 (amino acid sequence)

```
1 ATGGCCTGGGCTCTGCTGCTCCTOACCCTCCTCACTCAGGACACAGGGTCTGGGCCGAT
1 M A W A L L L L T L L T Q D T G S W A D
61 ATCCAGCTGGTGGAGTCTGGGGGAGGTGTGGTACGGCCTGGGGGTCCCTGAGACTCTCC
21 I Q L V E S G G G V V R P G G S L R L S
121 TGTGCAGCCTCTGGATTACCTTTGATGATTATGGCATGAGCTGGGTCCGCCAAGCTCCA
41 C A A S G F T F D D Y G M S W V R Q A P
181 GGAAGGGGCTGGAGTGGGTCTCTGGTATTAATTGGAATGGTGGTAGCACAGGTTATGCA
61 G K G L E W V S G I N W N G G S T G Y A
241 GACTCTGTGAAGGGCCGATTACCATCTCTAGAGACAACGCCAAGAAGTCCCTGTATCTG
81 D S V K G R F T I S R D N A K N S L Y L
301 - CAAATGAACAGTCTGAGAGCCGAGGACACGGCCGTGTATTACTGTGCAAGAATGAGGGCT
101 Q M N S L R A E D T A V Y Y C A R M R A
361 CCTGTGATTGGGGCCAAGGTACCCTGGTCACCGTCTCGAGTCTTCCACCAAGGGCCCA
121 P V I W G Q G T L V T V S S A S T K G P
421 TCGGTCTTCCCCCTGGCACCCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGC
141 S V F P L A P S S K S T S G G T A A L G
481 TGCCTGGTCAAGGACTACTTCCCGAACCAGGTGACGGTGTGCTGGAAGTCAAGGCGCCCTG
161 C L V K D Y F P E P V T V S W N S G A L
541 ACCAGCGCGGTGCACACCTTCCCGGCTGTCTACAGTCTCAGGACTCTACTCCCTCAGC
181 T S G V H T F P A V L Q S S G L Y S L S
601 AGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAAT
201 S V V T V P S S S L G T Q T Y I C N V N
661 CACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCCAAATCTTGTGACAAAAT
221 H K P S N T K V D K R V E P K S C D K T
721 CACACATGCCCACCGTGCCAGCACCTGAACTCCTGGGGGACTGTCACTCTTCOTCTTC
241 H T C P P C P A P E L L G G P S V F L F
781 CCCCCAAAACCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTACATGCGTGGTG
261 P P K P K D T L M I S R T P E V T C V V
841 GTGGACGTGAGCCACGAAGACCCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAG
281 V D V S H E D P E V K F N W Y V D G V E
901 GTGCATAATGCCAAGACAAAGCCGCGGAGGAGCAGTACAACAGCACGTACCGTGTGGTC
301 V H N A K T K P R E E Q Y N S T Y R V V
961 AGCGTCTCACCCTCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTC
321 S V L T V L H Q D W L N G K E Y K C K V
1021 TCCAACAAAGCCCTCCCGAGCCCCATCGAGAAAACCATCTCCAAGCCAAAGGGCAGCCC
341 S N K A L P A P I E K T I S K A K G Q P
1081 OGAGAACCACAGGTGTACACCCTGCCCCATCCCGGAGGAGATGACCAAGAACCAGGTC
361 R E P Q V Y T L P P S R E E M T K N Q V
1141 AGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCGAGCAGATCGCCGTGGAGTGGGAGAGC
381 S L T C L V K G F Y P S D I A V E W E S
1201 AATGGGCAGCCGGAGAACAATAACAAGACCACGTCTCCCGTGGTGGACTCCGACGGCTCC
401 N G Q P E N N Y K T T S P V L D S D G S
1261 TTCTTCTCTATAGCAAGCTCACCGTGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTC
421 F F L Y S K L T V D K S R W Q Q G N V F
1321 TCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTG
441 S C S V M H E A L H N H Y T Q K S L S L
1381 TCTCTGGGTAAATGA
461 S L G K *
```





FIG. 48B: The ORF and Amino Acid Sequence of Y1-LC

SEQ ID NO: 207 (nucleic acid sequence); SEQ ID NO: 208 (amino acid sequence)

```
1      ATGGCCTGGGCTCTGCTGCTCCTCACCCTCCTCACTCAGGACACAGGGTCCTGGGCCGAT
1      M A W A L L L L T L L T Q D T G S W A D
61     GCAGAGCTGACTCAGGACCCTGCTGTGTCTGTGGCCTTGGGACAGACAGTCAGGATCACA
21     A E L T Q D P A V S V A L G Q T V R I T
1212   TGCCAAGGAGACAGCCTCAGAAGCTATTATGCAAGCTGGTACCAGCAGAAGCCAGGACAG
41     C Q G D S L R S Y Y A S W Y Q Q K P G Q
181    GCCCCTGTA CTGTGTCATCTATGGTAAAAACAACCGGCCCTCAGGGATCCCAGACCGATT
161    A P V L V I Y G K N N R P S G I P D R F
241    TCTGGCTCCAGCTCAGGAAACACAGCTTCCTTGACCATCACTGGGGCTCAGGCGGAAGAT
81     S G S S S G N T A S L T I T G A Q A E D
301    GAGGCTGACTATTACTGTAAC TCCCGGGACAGCAGTGGAACCATGTGGTATTCCGGCGGA
101    E A D Y Y C N S R D S S G N H V V F G G
361    GGGACCAAGCTGACCGTCCTAGGTCAGCCCAAGGCTGCCCCCTCGGTCACTCTGTTCCCG
121    G T K L T V L G Q P K A A P S V T L F P
421    CCCTCCTCTGAGGAGCTTCAAGCCAACAAGGCCACACTGGTGTGTCTCATAAGTGACTTC
141    P S S E E L Q A N K A T L V C L I S D F
481    TACCCGGGAGCCGTGACAGTGGCCTGGAAGGCAGATAGCAGCCCCGTCAAGGCGGGAGTG
161    Y P G A V T V A W K A D S S P V K A G V
541    GAGACCACACACCCTCCAAACAAAGCAACAACAAGTACGCGGCCAGCAGCTACCTGAGC
181    E T T T P S K Q S N N K Y A A S S Y L S
601    CTGACGCCTGAGCAGTGGAAGTCCCACAAAAGCTACAGCTGCCAGGTACGCATGAAGGG
201    L T P E Q W K S H K S Y S C Q V T H E G
661    AGCACCGTGGAGAAGACAGTGGCCCCTACAGAATGTTTCATGA
221    S T V E K T V A P T E C S *
```



**FIG. 49**

1	11	21	31	41	51		
1	EVQLVESGGG	LVQPGGSLRL	SCAASGFTFS	SYAMSWVRQA	PGKGLEWVSA	ISGSGGSTYY	60
61	ADSVKGRFTI	SRDNSIKNTLY	LOMNSLRAED	TAVYYCARVA	KTLMRQYSLW	GQGTLVTVSR	120
121	GGGSGGGGGS	GGGGSSELTQ	DPAVSVALGQ	TVRITCGDS	LRSYIASWYQ	QKPGQAPVLV	180
181	IYGKNNRPSG	IPDRFSGSSS	GNTASLTITG	AQAEDEADYY	CNSRDSSGNH	VVFGGGTKLT	240
241	VLGAAAEQKL	ISEEDLNGAA					



FIG. 50

	10	20	30	40	50	60
1	AttTattTACTC	gGGGCCAGC	CgGCCAGC	CGAGGTGCAG	CTGGTGGAGT	CTGGGGGAGG
3	L L L A A Q P A M A	E V Q L V E S G G G				
	70	80	90	100	110	120
1	CTTGGTACAG	CCTGGGGGGT	CCCTGAGACT	CTCCTGTGCA	GCCTCTGGAT	TCACCTTTAG
3	L V Q P G G S L R L S C A A S G F T F S					
	130	140	150	160	170	180
1	CAGCTATGCC	ATGAGCTGGG	TCCGCCAGGC	TCCAGGGAAG	GGGCTGGAGT	GGGTCTCAGC
3	S Y A M S W V R Q A P G K G L E W V S A					
	190	200	210	220	230	240
1	TATTAGTGGT	AGTGGTGGTA	GCACATACTA	CGCAGACTCC	GTGAAGGGCC	GGTTCACCAT
3	F S G S G G S T Y Y A D S V K G R F T I					
	250	260	270	280	290	300
1	CTCCAGAGAC	AATTCGAAGA	ACAGCCTGTA	TCTGCAATG	AACAGCCTGA	GAGCCGAGGA
3	S R D N S K N T L Y L Q M N S L R A E D					
	310	320	330	340	350	360
1	CACGGCCGTG	TATTACTGTG	CAAGACGGG	CGCAGTATT	AGGGTAACT	GGGGCCAGG
3	T A V Y Y C A R T G Q S I K R S W G Q G					
	370	380	390	400	410	420
1	TACCCTGGTC	ACCGTGTGGA	GAGGTGGAGG	CGGTTCAggC	GGagGTGgCT	CTGGCGGTGG
3	T L V T V S R G G G G S G G G G S G G G					
	430	440	450	460	470	480
1	CGGATGCTCT	GAGCTGACTC	AGGACCCTGC	TGTGTCTGTG	GcCTTGGGAC	AgACAGTCAG
3	G S S E L T Q D P A V S V A L G Q T V R					
	490	500	510	520	530	540
1	GATCACATGC	CAAGGAGACA	GCCTCAGAAG	CTATTATGCA	AGCTGGTACC	AGCAGAAGCC
3	I T C Q G D S L R S Y Y A S W Y Q Q K P					
	550	560	570	580	590	600
1	AGGACAGGCC	CCTGTACTTG	TCATCTATGG	TAAAAACAAC	CGGCCCTCAG	GGATCCCAGA
3	G Q A P V L V I Y G K N N R P S G I P D					
	610	620	630	640	650	660
1	CGGATTCTCT	GGCTCCAGCT	CAGGAACAC	AGCTTCCTTG	ACCATCACTG	GGGCTCAGGC
3	R F S G S S S G N T A S L T I T G A Q A					
	670	680	690	700	710	720
1	GGAGATGAG	GCTGACTATT	ACTGTAATC	CCGGGACAGC	AGTGGTAACC	ATGTGGTATT
3	E D E A D Y Y C N S R D S S G N H V V F					
	730	740	750	760	770	780
1	CGGCGGAGGG	ACCAAGCTGA	CCGTCCTAGG	TGCGGCCGCA	GAACAAAAC	TCATCTCAGA
3	G G G T K L T V L G A A A E Q K L I S E					
	790	800	810	820	830	840
1	AGAGGatCTG	AatGGGGCCG	CAGGAACTG	TtGAATTTT	TAAGTAAcC	T
3	E D L N G A A * N C * I F * V N					

1/16 SEQ ID NO: 210



## ***FIG. 51***

Sequence of Y1-Biotag (SEQ ID NO: 211)

1 MEVQLVESGG GVVRPGGSLR LSCAASGFTF DDYGMSWVRQ  
41 APGKGLEWVS GINWNGGSTG YADSVKGRFT ISRDNAKNSL  
81 YLQMNSLRAE DTAVYYCARM RAPVIWGQGT LVTVSRGGGG  
121 SGGGGSGGGG SSELTDPAV SVALGQTVRI TCQGDSLRSY  
161 YASWYQQKPG QAPVLVIYGK NNRPSGIPDR FSGSSSGNTA  
201 SLTITGAQAE DEADYYCNSR DSSGNNVVFG GGTKLTVLGG  
241 GGLNDIFEAQ KIEWHE



## ***FIG. 52***

Y1-cys-kak scFv (SEQ ID NO. 212)

I MEVQLVESGG GVVVRPGGSLR LSCAASGFTF DDYGMSWVRQ  
APGKGLEWVS GINWNGGSTG 60

61 YADSVKGRFT ISRDNAKNSL YLQMNSLRAE DTAVYYCARM  
RAPVIWGQGT LVTVSRGGGG 120

121 SGGGSGGGG SSELTDPAV SVALGQTVRI TCQGDSLRSY  
YASWYQQKPG QAPVLVIYGK 180

181 NNRPSGIPDR FSGSSSGNTA SLTITGAQAE DEADYYCNSR  
DSSGNHVVFG GGTKLTVLGG 240

241 GGCKAK